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(54) Title: ENDO BETA-1,4-GLUCANASE FROM ASPERGILLUS (57) Abstract A glucanase enzyme is described. In addition, there is described a nucleotide sequence coding for the same and a promoter for controlling the expression of the same.		

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ENDO BETA-1,4-GLUCANASE FROM ASPERGILLUS

The present invention relates to an enzyme. In addition, the present invention relates to a nucleotide sequence coding for the enzyme. Also, the present invention relates to a promoter, wherein the promoter can be used to control the expression of the nucleotide sequence coding for the enzyme.

In particular, the enzyme of the present invention is a glucanase enzyme - i.e. an enzyme that can degrade β -1,4-glucosidic bonds.

It is known that it is desirable to direct expression of a gene of interest ("GOI") in certain tissues of an organism - such as a filamentous fungus (such as *Aspergillus niger*) or even a plant crop. The resultant protein or enzyme may be useful for the organism itself. For example, it may be desirable to produce crop protein products with an optimised amino acid composition and so increase the nutritive value of a crop. For example, the crop may be made more useful as a feed. In the alternative, it may be desirable to isolate the resultant protein or enzyme and then use the protein or enzyme to prepare, for example, food compositions. In this regard, the resultant protein or enzyme can be a component of the food composition or it can be used to prepare food compositions, including altering the characteristics or appearance of food compositions.

It may even be desirable to use the organism, such as a filamentous fungus or a crop plant, to express non-plant genes, such as for the same purposes.

Also, it may be desirable to use an organism, such as a filamentous fungus or a crop plant, to express mammalian genes. Examples of the latter products include interferons, insulin, blood factors and plasminogen activators.

It is also desirable to use micro-organisms, such as filamentous fungi, to prepare products from GOIs by use of promoters that are active in the micro-organisms.

Fruit and vegetable cell walls largely consist of polysaccharide, the major components being pectin, cellulose and xyloglucan, R.R. Selvendran and J.A. Robertson, IFR Report 1989. Numerous cell wall models have been proposed which attempt to incorporate the essential properties of strength and flexibility (P. Albersheim, Sci. Am. 232, 81-95, 1975; P. Albersheim, Plant Biochem. 3rd Edition (Bonner and Varner), Ac. Press, 1976; T. Hayashi, Ann. Rev. Plant Physiol. & Plant Mol. Biol., 40, 139-168, 1989).

The composition of the plant cell wall is complex and variable. Polysaccharides are mainly found in the form of long chains of cellulose (the main structural component of the plant cell wall), hemicellulose (comprising various β -xylan chains, such as xyloglucans) and pectic substances (consisting of galacturonans and rhamnogalacturonans; arabinans; and galactans and arabinogalactans).

In particular, glucans are polysaccharides made up exclusively of glucose subunits. Typical examples of glucans are starch and cellulose.

The enzymes that degrade glucans are collectively referred to as glucanases. A typical glucanase is β -1,4-endoglucanase.

β -1,4-endoglucanases have uses in many industries. For example, in the brewing industry, barley is used for production of malt, and, in the latter years, as adjunct in the brewing process. When the quality of the malt is poor, or barley has been used as an adjunct, problems with high viscosity in the wort can arise because of β -glucans from the barley. In this regard, barley contains large quantities of mixed β -1,3/1,4- glucans of very high molecular weight. When dissolved, these glucans produce high viscosity solutions, which can cause troubles in some applications. For example, the high viscosity reduces the filterability of the wort and can lead to unacceptable long filtration times. To avoid these problems β -glucanase has been traditionally added to wort to avoid such problems - i.e. the problem with glucans can be avoided by addition of enzymes. In particular, glucanases, which degrade the polymers.

Further information on these problems may be found in the Grindsted brochure called "Glucanase GV", the reviews by Dr. C.W. Bamforth (Brewers Digest June 1982 pages 22-28; and Brewers' Guardian September 1985 pages 21-26), and the paper by T. Godfrey (Industrial Enzymology The Application of Enzymes in Industry Chapter 4.5 pages 221-259).

In the feed industry barley can be used for chicken feed because it is cheap, but again the β -glucan can give problems for the digestion of the chicken. By addition of β -glucanase to the feed the digestibility of the feed can be increased. In addition, the faeces of chickens feeding on feed containing barley is sticky making it difficult to remove and results in dirty eggs.

WO 93/2019 discusses endo- β -1,4-glucanases (EC no. 3.2.1.4). According to WO 93/2019, these glucanases are a group of hydrolases which catalyse endo hydrolysis of 1,4- β -D-glycosidic linkages in cellulose, lichenin, cereal β -D-glucans and other plant material containing cellulosic parts. Endo-1,4- β -D-glucan 4-glucano hydrolase is sometimes called endo- β -1,4-glucanase.

The endo- β -1,4-glucanase of WO 93/2019 exhibits a pH-optimum of 2.0 to 4.0, an isoelectric point of 2.0 to 3.5, a molecular weight of between 30,000 and 50,000, and a temperature optimum between 30 and 70°C.

Further teachings on glucans may be found in WO 93/17101, in particular xyloglucans. According to WO 93/17101 the xyloglucans are 1,4- β -glucans that have been extensively substituted with α -1,6-xylosyl side chains, some of which are 1,2- β -galactosylated. They are found in large amounts in the primary cell walls of dicots but also in certain seeds, where they serve different roles. Primary cell wall xyloglucan is fucosylated. Xyloglucan is tightly hydrogen bonded to cellulose microfibrils and requires concentrated alkali or strong swelling agents to release it. Xyloglucan is thought to form cross-bridges between cellulose microfibrils, the cellulose/xyloglucan network forming the major load-bearing/elastic network of the wall. DCB mutated suspension culture cells (cell walls lacking cellulose) release xyloglucan into their media, suggesting that xyloglucan is

normally rightly bound to cellulose.

Hydrolysis of primary cell wall xyloglucan has been demonstrated in segments of dark grown squash hypocotyls, during IAA induced growth (K. Wakabayashi et al, Plant
5 Physiol., 95, 1070-1076, 1991). Endohydrolysis of wall xyloglucan is thought to contribute to wall loosening which accompanies cell expansion (T. Hyashi, Ann. Rev. Plant Physiol. & Plant Mol. Biol., 40, 139-168, 1989). The average molecular weight of xyloglucan has also been shown to decrease during tomato fruit ripening and this may contribute to the tissue softening which accompanies the ripening process (D.J. Huber,
10 J. Amer. Soc. Hort. Sci., 108(3), 405-409, 1983). Certain seeds, e.g. Nasturtium, contain up to 30% by weight of xyloglucan, stored in thickened cotyledonary cell walls, which serves as a reserve polysaccharide and is rapidly depolymerised during germination.

15 It would be useful to increase glucanase activity, for example to have a plant with high concentration of glucanase for use in feed, preferably using recombinant DNA techniques.

The present invention seeks to provide an enzyme having glucanase activity; preferably
20 wherein the enzyme can be prepared in certain or specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*, or even a plant.

Also, the present invention seeks to provide a GOI coding for the enzyme that can be
25 expressed preferably in specific cells or tissues, such as in certain or specific cells or tissues, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*, or even a plant.

In addition, the present invention seeks to provide a promoter that is capable of directing
30 expression of a GOI, such as a nucleotide sequence coding for the enzyme according to the present invention, preferably in certain specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the

genus *Aspergillus*, such as *Aspergillus niger*, or even a plant. Preferably, the promoter is used in *Aspergillus* wherein the product encoded by the GOI is excreted from the host organism into the surrounding medium.

5 Furthermore, the present invention seeks to provide constructs, vectors, plasmids, cells, tissues, organs and organisms comprising the GOI and/or the promoter, and methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, or even a plant.

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According to a first aspect of the present invention there is provided an enzyme obtainable from *Aspergillus*, wherein the enzyme has the following characteristics: a-MW of 24,235 D \pm 50 D; a pI value of about 4; glucanase activity; and wherein the glucanase activity is endo β -1,4-glucanase activity.

15

According to a second aspect of the present invention there is provided an enzyme having the sequence shown as SEQ. I.D. No. 1 or a variant, homologue or fragment thereof.

20 According to a third aspect of the present invention there is provided an enzyme coded by the nucleotide sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

According to a fourth aspect of the present invention there is provided a nucleotide sequence coding for the enzyme according to the present invention.

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According to a fifth aspect of the present invention there is provided a nucleotide sequence having the sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

30 According to a sixth aspect of the present invention there is provided a promoter having the sequence shown as SEQ. I.D. No. 3 or a variant, homologue or fragment thereof or a sequence complementary thereto.

According to a seventh aspect of the present invention there is provided a terminator having the nucleotide sequence shown as SEQ. I.D. No. 13 or a variant, homologue or fragment thereof or a sequence complementary thereto.

- 5 According to an eighth aspect of the present invention there is provided a signal sequence having the nucleotide sequence shown as SEQ. I.D. No. 14 or a variant, homologue or fragment thereof or a sequence complementary thereto.

- 10 According to a ninth aspect of the present invention there is provided a process for expressing a GOI by use of a promoter, wherein the promoter is the promoter according to the present invention.

- 15 According to a tenth aspect of the present invention there is provided the use of an enzyme according to the present invention to degrade a glucan.

- 20 According to an eleventh aspect of the present invention there is provided plasmid NCIMB 40704, or a nucleotide sequence obtainable therefrom for expressing an enzyme capable of degrading arabinoxylan or for controlling the expression thereof or for controlling the expression of another GOI.

- 25 According to a twelfth aspect of the present invention there is provided a signal sequence having the sequence shown as SEQ. I.D. No. 15 or a variant, homologue or fragment thereof.

- 30 According to a thirteenth aspect of the present invention there is provided a glucanase enzyme having the ability to degrade β -1,4-glucosidic bonds, which is immunologically reactive with an antibody raised against a purified glucanase enzyme having the sequence shown as SEQ. I.D. No. 1.

- 35 According to a fourteenth aspect of the present invention there is provided a promoter that is inducible by glucose.

According to a fifteenth aspect of the present invention there is provided the use of glucose to induce a promoter.

5 Other aspects of the present invention include constructs, vectors, plasmids, cells, tissues, organs and transgenic organisms comprising the aforementioned aspects of the present invention.

10 Other aspects of the present invention include methods of expressing or allowing expression or transforming any one of the nucleotide sequence, the construct, the plasmid, the vector, the cell, the tissue, the organ or the organism, as well as the products thereof.

15 Additional aspects of the present invention include uses of the promoter for expressing GOIs in culture media such as a broth or in a transgenic organism.

Further aspects of the present invention include uses of the enzyme for preparing or treating foodstuffs, including animal feed.

20 In the following text, the enzyme of the present invention is sometimes referred to as Egla enzyme and the coding sequence therefor is sometimes referred to as the Egla gene. In addition, the promoter of the present invention is sometimes referred to as Egla promoter.

25 Preferably the enzyme is coded by the nucleotide sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

Preferably the nucleotide sequence has the sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

30 Preferably the nucleotide sequence is operatively linked to a promoter.

Preferably the promoter is the promoter having the sequence shown as SEQ. I.D. No. 3 or a variant, homologue or fragment thereof or a sequence complementary thereto.

Preferably the promoter of the present invention is operatively linked to a GOI.

5

Preferably the GOI comprises a nucleotide sequence according to the present invention.

In one preferred embodiment, the transgenic organism is a fungus. For example the organism can be a yeast, which would then be useful in for example the brewing industry.

10

Preferably the transgenic organism is a filamentous fungus, more preferably of the genus *Aspergillus*.

15 In another preferred embodiment the transgenic organism is a plant.

In another preferred embodiment the transgenic organism is a yeast. In this regard, yeast have been widely used as a vehicle for heterologous gene expression. The species *Saccharomyces cerevisiae* has a long history of industrial use, including use for heterologous gene expression. Expression of heterologous genes in *Saccharomyces cerevisiae* has been reviewed by Goodey *et al* (1987, Yeast Biotechnology, D R Berry *et al*, eds, pp 401-429, Allen and Unwin, London) and by King *et al* (1989, Molecular and Cell Biology of Yeasts, E F Walton and G T Yarronton, eds, pp 107-133, Blackie, Glasgow).

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For several reasons *Saccharomyces cerevisiae* is well suited for heterologous gene expression. First, it is non-pathogenic to humans and incapable of producing certain endotoxins. Second, it has a long history of safe use following centuries of commercial exploitation for various purposes. This has led to wide public acceptability. Third, the extensive commercial use and research devoted to the organism has resulted in a wealth of knowledge about the genetics and physiology as well as large-scale fermentation characteristics of *Saccharomyces cerevisiae*.

30

An additional advantage is that yeasts are capable of post-translational modifications of proteins and thereby have the potential for glycosylation and/or secretion of heterologous gene products into the growth medium. A review of the principles of heterologous gene expression in *Saccharomyces cerevisiae* and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for the expression of heterologous genes", Yeasts, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).

The glycosylation of enzymes expressed in yeast is known to increase heat stability of the enzyme. Enhancing the heat stability of the glucanase according to the present invention will make this enzyme suitable for use in the brewing industry and for use in the preparation of animal feed, i.e. chicken feed.

Yeasts are known to secrete very few proteins into the culture medium. This makes yeast a very attractive host for expression of heterologous genes, since secretable gene products can easily be recovered and purified.

Several types of yeast vectors are available, including integrative vectors, which require recombination with the host genome for their maintenance, and autonomously replicating plasmid vectors.

In order to prepare the transgenic *Saccharomyces*, expression constructs are prepared by inserting a GOI (such as an amylase or SEQ. ID No 2) into a construct designed for expression in yeast. Several types of constructs used for heterologous expression have been developed. The constructs contain a promoter active in yeast fused to the GOI, usually a promoter of yeast origin, such as the GAL1 promoter, is used. The GOI can be fused to a signal sequence which directs the protein encoded by the GOI to be secreted. Usually a signal sequence of yeast origin, such as the sequence encoding the SUC2 signal peptide, is used. A terminator active in yeast ends the expression system.

30

Heterologous expression in yeast has been reported for several genes. The gene products can be glycosylated which is advantageous for some enzymes intended for specific

application where heat tolerance is desirable. The proteins can be deposited intracellularly if the GOI is not fused to a signal sequence, or they can be secreted extracellularly if the GOI is equipped with a signal sequence.

5 For the transformation of yeast several transformation protocols have been developed.

For example, the transgenic *Saccharomyces* according to the present invention can be prepared by following the teachings of Hinnen *et al* (1978, Proceedings of the National Academy of Sciences of the USA 75, 1929) Beggs, J D (1978, Nature, London, 275,
10 104); and Ito, H *et al* (1983, J Bacteriology 153, 163-168).

The transformed yeast cells are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as LEU2, HIS4 and TRP1, and dominant antibiotic resistance markers such as aminoglycoside
15 antibiotic markers, eg G418.

Highly preferred embodiments of each of the aspects of the present invention do not include any one of the native enzyme, the native promoter or the native nucleotide sequence in its natural environment.

20

Preferably, in any one of the plasmid, the vector such as an expression vector or a transformation vector, the cell, the tissue, the organ, the organism or the transgenic organism, the promoter is present in combination with at least one GOI.

25 Preferably the promoter and the GOI are stably incorporated within the transgenic organism's genome.

Preferably the transgenic organism is a filamentous fungus, preferably of the genus *Aspergillus*, more preferably *Aspergillus niger*. Alternatively, the transgenic organism
30 can be a yeast. The transgenic organism can even be a plant, such as a monocot or dicot plant.

A highly preferred embodiment is an enzyme obtainable from *Aspergillus*, wherein the enzyme has the following characteristics: a MW of 24,235 D \pm 50 D; a pI value of about 4; glucanase activity; and wherein the glucanase activity is endo β -1,4-glucanase activity; wherein the enzyme has the sequence shown as SEQ. I.D. No. 1 or a variant, 5
homologue or fragment thereof.

Another highly preferred embodiment is an enzyme obtainable from *Aspergillus*, wherein the enzyme has the following characteristics: a MW of 24,235 D \pm 50 D; a pI value of about 4; glucanase activity; and wherein the glucanase activity is endo β -1,4-glucanase 10
activity; wherein the enzyme is coded by the nucleotide sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.

The advantages of the present invention are that it provides a means for preparing a glucanase enzyme and the nucleotide sequence coding for the same. In addition, it 15
provides a promoter that can control the expression of that, or another, nucleotide sequence.

Other advantages of the present invention are that the enzyme can be used to prepare useful feeds containing cereals, such as barley, maize, rice etc.

20
The present invention therefore provides an enzyme having glucanase activity wherein the enzyme can be prepared in certain or specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*. The enzyme may even be prepared in a 25
plant.

Also, the present invention provides a GOI coding for the enzyme that can be expressed preferably in specific cells or tissues, such as in certain or specific cells or tissues, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, such as 30
Aspergillus niger. The GOI may even be expressed in a plant.

In addition, the present invention provides a promoter that is capable of directing expression of a GOI, such as a nucleotide sequence coding for the enzyme according to the present invention, preferably in certain specific cells or tissues, such as in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the
5 genus *Aspergillus*, such as *Aspergillus niger*, or even a plant. Preferably, the promoter is used in *Aspergillus* wherein the product encoded by the GOI is excreted from the host organism into the surrounding medium. The promoter may even be tailored (if necessary) to express a GOI in a plant.

10 The present invention also provides constructs, vectors, plasmids, cells, tissues, organs and organisms comprising the GOI and/or the promoter, and methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a filamentous fungus, preferably of the genus *Aspergillus*, or even a plant.

15 The terms "variant", "homologue" or "fragment" in relation to the enzyme include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant amino acid sequence has glucanase activity, preferably having at least the same activity of the
20 enzyme shown in the sequence listings (SEQ I.D. No. 1 or 12). In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant enzyme has glucanase activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO. 1 shown in the attached sequence listings. More preferably
25 there is at least 95%, more preferably at least 98%, homology to SEQ ID NO. 1 shown in the attached sequence listings.

The terms "variant", "homologue" or "fragment" in relation to the nucleotide sequence coding for the enzyme include any substitution of, variation of, modification of,
30 replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for an enzyme having glucanase activity, preferably having at least the same activity of the enzyme shown in

the sequence listings (SEQ I.D. No. 2 or 12). In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence codes for an enzyme having glucanase activity. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO. 2 shown in the attached sequence listings. More preferably there is at least 95%, more preferably at least 98%, homology to SEQ ID NO. 2 shown in the attached sequence listings.

The terms "variant", "homologue" or "fragment" in relation to the promoter include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence has the ability to act as a promoter in an expression system - such as the transformed cell or the transgenic organism according to the present invention. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence has the ability to act as a promoter. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO. 3 shown in the attached sequence listings. More preferably there is at least 95%, more preferably at least 98%, homology to SEQ ID NO. 3 shown in the attached sequence listings.

The terms "variant", "homologue" or "fragment" in relation to the terminator or signal nucleotide sequences include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence has the ability to act as a terminator or codes for an amino acid sequence that has the ability to act as a signal sequence respectively in an expression system - such as the transformed cell or the transgenic organism according to the present invention. In particular, the term "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence has the ability to act as or code for a terminator or signal respectively. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO.s 13 and 14 (respectively) shown in the attached sequence listings. More preferably there is at least 95%, more

preferably at least 98%, homology to SEQ ID NO.s 13 and 14 (respectively) shown in the attached sequence listings.

5 The terms "variant", "homologue" or "fragment" in relation to the signal amino acid sequence include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant sequence has the ability to act as a signal sequence in an expression system - such as the transformed cell or the transgenic organism according to the present invention. In particular, the term "homologue" covers homology with respect to structure
10 and/or function providing the resultant nucleotide sequence has the ability to act as or code for a signal. With respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to SEQ ID NO 15 shown in the attached sequence listings. More preferably there is at least 95%, more preferably at least 98%, homology to SEQ ID NO 15 shown in the attached sequence
15 listings.

The above terms are synonymous with allelic variations of the sequences.

20 The term "complementary" means that the present invention also covers nucleotide sequences that can hybridise to the nucleotide sequences of the coding sequence, the promoter sequence, the terminator sequence or the signal sequence respectively.

The term "nucleotide" in relation to the present invention includes genomic DNA, CDNA, synthetic DNA, and RNA. Preferably it means DNA, more preferably cDNA
25 for the coding sequence of the present invention since the genomic coding sequence has two introns and their removal would allow expression in bacteria.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - includes a GOI directly or indirectly attached to a promoter. An example
30 of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the *Sh1*-intron or the ADH intron, intermediate the promoter and the GOI. The same is true for the term "fused" in relation to the present invention which

includes direct or indirect attachment. In each case, it is highly preferred that the terms do not cover the natural combination of the gene coding for the enzyme ordinarily associated with the wild type gene promoter and when they are both in their natural environment. A highly preferred embodiment is the or a GOI being operatively linked
5 to a or the promoter.

The construct may even contain or express a marker which allows for the selection of the genetic construct in, for example, a filamentous fungus, preferably of the genus *Aspergillus*, such as *Aspergillus niger*, or plants, preferably cereals, such as maize, rice,
10 barley etc., into which it has been transferred. Various markers exist which may be used, such as for example those encoding mannose-6-phosphate isomerase (especially for plants) or those markers that provide for antibiotic resistance - e.g. resistance to G418, hygromycin, bleomycin, kanamycin and gentamycin.

15 The term "vector" includes expression vectors and transformation vectors.

The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression.

The term "transformation vector" means a construct capable of being transferred from
20 one species to another - such as from an *E.coli* plasmid to a filamentous fungus, preferably of the genus *Aspergillus*. It may even be a construct capable of being transferred from an *E.coli* plasmid to an *Agrobacterium* to a plant.

The term "tissue" includes tissue *per se* and organ.

25

The term "organism" in relation to the present invention includes any organism that could comprise the promoter according to the present invention and/or the nucleotide sequence coding for the enzyme according to the present invention and/or products obtained therefrom, wherein the promoter can allow expression of a GOI and/or wherein the
30 nucleotide sequence according to the present invention can be expressed when present in the organism.

Preferably the organism is a filamentous fungus, preferably of the genus *Aspergillus*, more preferably *Aspergillus niger*.

5 The term "transgenic organism" in relation to the present invention includes any organism that comprises the promoter according to the present invention and/or the nucleotide sequence coding for the enzyme according to the present invention and/or products obtained therefrom, wherein the promoter can allow expression of a GOI and/or wherein the nucleotide sequence according to the present invention can be expressed within the organism. Preferably the promoter and/or the nucleotide sequence is (are) incorporated
10 in the genome of the organism. Preferably the transgenic organism is a filamentous fungus, preferably of the genus *Aspergillus*, more preferably *Aspergillus niger*.

Therefore, the transgenic organism of the present invention includes an organism comprising any one of, or combinations of, the promoter according to the present
15 invention, the nucleotide sequence coding for the enzyme according to the present invention, constructs according to the present invention, vectors according to the present invention, plasmids according to the present invention, cells according to the present invention, tissues according to the present invention or the products thereof. For example the transgenic organism can comprise a GOI, preferably an exogenous nucleotide
20 sequence, under the control of the promoter according to the present invention. The transgenic organism can also comprise the nucleotide sequence coding for the enzyme of the present invention under the control of a promoter, which may be the promoter according to the present invention.

25 In a highly preferred embodiment, the transgenic organism does not comprise the combination of the promoter according to the present invention and the nucleotide sequence coding for the enzyme according to the present invention, wherein both the promoter and the nucleotide sequence are native to that organism and are in their natural environment. Thus, in these highly preferred embodiments, the present invention does
30 not cover the native nucleotide coding sequence according to the present invention in its natural environment when it is under the control of its native promoter which is also in its natural environment. In addition, in this highly preferred embodiment, the present

invention does not cover the native enzyme according to the present invention when it is in its natural environment and when it has been expressed by its native nucleotide coding sequence which is also in its natural environment and when that nucleotide sequence is under the control of its native promoter which is also in its natural environment.

5

The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site in the Jacob-Mond theory of gene expression.

10

In one aspect, the promoter of the present invention is capable of expressing a GOI, which can be the nucleotide sequence coding for the enzyme of the present invention.

15

In another aspect, the nucleotide sequence according to the present invention is under the control of a promoter that allows expression of the nucleotide sequence. In this regard, the promoter need not necessarily be the same promoter as that of the present invention. In this aspect, the promoter may be a cell or tissue specific promoter. If, for example, the organism is a plant then the promoter can be one that affects expression of the nucleotide sequence in any one or more of stem, sprout, root and leaf tissues.

20

By way of example, the promoter for the nucleotide sequence of the present invention can be the α -Amy 1 promoter (otherwise known as the Amy 1 promoter, the Amy 637 promoter or the α -Amy 637 promoter) as described in our co-pending UK patent application No. 9421292.5 filed 21 October 1994. That promoter comprises the sequence shown in Figure 1.

25

Alternatively, the promoter for the nucleotide sequence of the present invention can be the α -Amy 3 promoter (otherwise known as the Amy 3 promoter, the Amy 351 promoter or the α -Amy 351 promoter) as described in our co-pending UK patent application No. 9421286.7 filed 21 October 1994. That promoter comprises the sequence shown in Figure 2.

30

Preferably, the promoter is the promoter of the present invention.

In addition to the nucleotide sequences described above, the promoters, particularly that of the present invention, could additionally include features to ensure or to increase expression in a suitable host. For example, the features can be conserved regions such as a Pribnow Box or a TATA box. The promoters may even contain other sequences to affect (such as to maintain, enhance, decrease) the levels of expression of the GOI. For example, suitable other sequences include the *Shl*-intron or an ADH intron. Other sequences include inducible elements - such as temperature, chemical, light or stress inducible elements. Also, suitable elements to enhance transcription or translation may be present. An example of the latter element is the TMV 5' signal sequence (see Sleat Gene 217 [1987] 217-225; and Dawson Plant Mol. Biol. 23 [1993] 97).

In addition the present invention also encompasses combinations of promoters and/or nucleotide sequences coding for proteins or enzymes and/or elements. For example, the present invention encompasses the combination of a promoter according to the present invention operatively linked to a GOI, which could be a nucleotide sequence according to the present invention, and another promoter such as a tissue specific promoter operatively linked to the same or a different GOI.

The present invention also encompasses the use of promoters to express a nucleotide sequence coding for the enzyme according to the present invention, wherein a part of the promoter is inactivated but wherein the promoter can still function as a promoter. Partial inactivation of a promoter in some instances is advantageous.

In particular, with the Amy 351 promoter mentioned earlier it is possible to inactivate a part of it so that the partially inactivated promoter expresses GOIs in a more specific manner such as in just one specific tissue type or organ.

The term "inactivated" means partial inactivation in the sense that the expression pattern of the promoter is modified but wherein the partially inactivated promoter still functions as a promoter. However, as mentioned above, the modified promoter is capable of expressing a GOI in at least one (but not all) specific tissue of the original promoter. One such promoter is the Amy 351 promoter described above.

Examples of partial inactivation include altering the folding pattern of the promoter sequence, or binding species to parts of the nucleotide sequence, so that a part of the nucleotide sequence is not recognised by, for example, RNA polymerase. Another, and preferable, way of partially inactivating the promoter is to truncate it to form fragments thereof. Another way would be to mutate at least a part of the sequence so that the RNA polymerase can not bind to that part or another part.

Another modification is to mutate the binding sites for regulatory proteins for example the CreA protein known from filamentous fungi to exert carbon catabolite repression, and thus abolish the catabolite repression of the native promoter.

The term "GOI" with reference to the present invention means any gene of interest. A GOI can be any nucleotide that is either foreign or natural to the organism (e.g. filamentous fungus, preferably of the genus *Aspergillus*, or a plant) in question. Typical examples of a GOI include genes encoding for proteins and enzymes that modify metabolic and catabolic processes. The GOI may code for an agent for introducing or increasing pathogen resistance. The GOI may even be an antisense construct for modifying the expression of natural transcripts present in the relevant tissues. The GOI may even code for a non-natural protein of a filamentous fungus, preferably of the genus *Aspergillus*, or a compound that is of benefit to animals or humans.

For example, the GOI could code for a pharmaceutically active protein or enzyme such as any one of the therapeutic compounds insulin, interferon, human serum albumin, human growth factor and blood clotting factors. In this regard, the transformed cell or organism could prepare acceptable quantities of the desired compound which would be easily retrievable from the cell or organism. The GOI may even be a protein giving nutritional value to a food or crop. Typical examples include plant proteins that can inhibit the formation of anti-nutritive factors and plant proteins that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant). The GOI may even code for an enzyme that can be used in food processing such as chymosin, thaumatin and α -galactosidase. The GOI can be a gene encoding for any one of a pest toxin, an antisense transcript such as that for patatin or α -amylase, ADP-glucose

pyrophosphorylase (e.g. see EP-A-0455316), a protease antisense or a glucanase.

The GOI can be the nucleotide sequence coding for the α -amylase enzyme which is the subject of our co-pending UK patent application 9413439.2 filed on 4 July 1994, the sequence of which is shown in Figure 3. The GOI can be the nucleotide sequence coding for the α -amylase enzyme which is the subject of our co-pending UK patent application 9421290.9 filed on 21 October 1994, the sequence of which is shown in Figure 4. The GOI can be any of the nucleotide sequences coding for the ADP-glucose pyrophosphorylase enzymes which are the subject of our co-pending PCT patent application PCT/EP94/01082 filed 7 April 1994, the sequences of which are shown in Figures 5 and 6. The GOI can be any of the nucleotide sequences coding for the α -glucan lyase enzyme which are described in our co-pending PCT patent application PCT/EP94/03397 filed 15 October 1994, the sequences of which are shown in Figures 7-10.

15

In one preferred embodiment, the GOI is a nucleotide sequence coding for the enzyme according to the present invention.

As mentioned above, a preferred host organism is of the genus *Aspergillus*, such as *Aspergillus niger*.

20

The transgenic *Aspergillus* according to the present invention can be prepared by following the teachings of Rambosek, J. and Leach, J. 1987 (Recombinant DNA in filamentous fungi: Progress and Prospects. CRC Crit. Rev. Biotechnol. 6:357-393), Davis R.W. 1994 (Heterologous gene expression and protein secretion in *Aspergillus*. In: Martinelli S.D., Kinghorn J.R. (Editors) *Aspergillus: 50 years on*. Progress in industrial microbiology vol 29. Elsevier Amsterdam 1994. pp 525-560), Ballance, D.J. 1991 (Transformation systems for Filamentous Fungi and an Overview of Fungal Gene structure. In: Leong, S.A., Berka R.M. (Editors) *Molecular Industrial Mycology. Systems and Applications for Filamentous Fungi*. Marcel Dekker Inc. New York 1991. pp 1-29) and Turner G. 1994 (Vectors for genetic manipulation. In: Martinelli S.D., Kinghorn J.R. (Editors) *Aspergillus: 50 years on*. Progress in industrial microbiology vol 29.

30

Elsevier Amsterdam 1994, pp. 641-666). However, the following commentary provides a summary of those teachings for producing transgenic *Aspergillus* according to the present invention.

5 Filamentous fungi have during almost a century been widely used in industry for production of organic compounds and enzymes. Traditional Japanese koji and soy fermentations have used *Aspergillus sp.* for hundreds of years. In this century *Aspergillus niger* has been used for production of organic acids particular citric acid and for production of various enzymes for use in industry.

10

There are two major reasons for that filamentous fungi have been so widely used in industry. First filamentous fungi can produce high amounts of extracellular products, for example enzymes and organic compounds such as antibiotics or organic acids. Second filamentous fungi can grow on low cost substrates such as grains, bran, beet pulp etc.

15 The same reasons have made filamentous fungi attractive organisms as hosts for heterologous expression according to the present invention.

In order to prepare the transgenic *Aspergillus*, expression constructs are prepared by inserting a GOI (such as an amylase or SEQ. I.D. No. 2) into a construct designed for
20 expression in filamentous fungi.

Several types of constructs used for heterologous expression have been developed. The constructs contain the promoter according to the present invention (or if desired another promoter if the GOI codes for the enzyme according to the present invention) which is
25 active in fungi. Examples of promoters other than that of the present invention include a fungal promoter for a highly expressed extracellular enzyme, such as the glucoamylase promoter or the α -amylase promoter. The GOI can be fused to a signal sequence (such as that of the present invention or another suitable sequence) which directs the protein encoded by the GOI to be secreted. Usually a signal sequence of fungal origin is used.
30 such as that of the present invention. A terminator active in fungi ends the expression system, such as that of the present invention.

Another type of expression system has been developed in fungi where the GOI is fused to a smaller or a larger part of a fungal gene encoding a stable protein. This can stabilize the protein encoded by the GOI. In such a system a cleavage site, recognized by a specific protease, can be introduced between the fungal protein and the protein encoded by the GOI, so the produced fusion protein can be cleaved at this position by the specific protease thus liberating the protein encoded by the GOI ("POI"). By way of example, one can introduce a site which is recognized by a KEX-2 like peptidase found in at least some *Aspergilli*. Such a fusion leads to cleavage *in vivo* resulting in protection of the POI and production of POI and not a larger fusion protein.

10

Heterologous expression in *Aspergillus* has been reported for several genes coding for bacterial, fungal, vertebrate and plant proteins. The proteins can be deposited intracellularly if the GOI is not fused to a signal sequence. Such proteins will accumulate in the cytoplasm and will usually not be glycosylated which can be an advantage for some bacterial proteins. If the GOI is equipped with a signal sequence the protein will accumulate extracellularly.

15

With regard to product stability and host strain modifications, some heterologous proteins are not very stable when they are secreted into the culture fluid of fungi. Most fungi produce several extracellular proteases which degrade heterologous proteins. To avoid this problem special fungal strains with reduced protease production have been used as host for heterologous production.

20

For the transformation of filamentous fungi, several transformation protocols have been developed for many filamentous fungi (Ballance 1991, *ibid*). Many of them are based on preparation of protoplasts and introduction of DNA into the protoplasts using PEG and Ca^{2+} ions. The transformed protoplasts then regenerate and the transformed fungi are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as *argB*, *trpC*, *niaD* and *pyrG*, antibiotic resistance markers such as benomyl resistance, hygromycin resistance and phleomycin resistance. A very common used transformation marker is the *amdS* gene of *A. nidulans* which in high copy number allows the fungus to grow with acrylamide as the sole

25

30

nitrogen source.

Even though the enzyme, the nucleotide sequence coding for same and the promoter of the present invention are not disclosed in EP-B-0470145 and CA-A-2006454, those two documents do provide some useful background commentary on the types of techniques that may be employed to put the present invention into practice. Some of these background teachings are now included in the following commentary.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material. Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system.

A review of the general techniques may be found in articles by Potrykus (*Annu Rev Plant Physiol Plant Mol Biol* [1991] 42:205-225) and Christou (*Agro-Food-Industry Hi-Tech* March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a promoter or nucleotide sequence or construct according to the present invention and which is capable of introducing the promoter or nucleotide sequence or construct into the genome of an organism, such as a plant.

The vector system may comprise one vector, but it can comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An et al. (1980), *Binary Vectors, Plant Molecular Biology Manual A3*, 1-19.

One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An et al. (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. et al. (1980), *Tissue Culture*

Methods for Plant Pathologists, eds.: D.S. Ingrams and J.P. Helgeson, 203-208.

Several different Ti and Ri plasmids have been constructed which are suitable for the construction of the plant or plant cell constructs described above. A non-limiting
5 example of such a Ti plasmid is pGV3850.

The promoter or nucleotide sequence or construct of the present invention should preferably be inserted into the Ti-plasmid between the terminal sequences of the T-DNA or adjacent a T-DNA sequence so as to avoid disruption of the sequences immediately
10 surrounding the T-DNA borders, as at least one of these regions appear to be essential for insertion of modified T-DNA into the plant genome.

As will be understood from the above explanation, if the organism is a plant, then the vector system of the present invention is preferably one which contains the sequences
15 necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct.

Furthermore, the vector system is preferably an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof, as these plasmids are
20 well-known and widely employed in the construction of transgenic plants, many vector systems exist which are based on these plasmids or derivatives thereof.

In the construction of a transgenic plant the promoter or nucleotide sequence or construct of the present invention may be first constructed in a microorganism in which the vector
25 can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli*, it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the promoter or nucleotide
30 sequence or construct of the invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the invention, which

DNA is subsequently transferred into the plant cell to be modified.

As reported in CA-A-2006454, a large amount of cloning vectors are available which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR 322, pUC series, M13 mp series, pACYC 184 etc. In such a way, the nucleotide or construct or promoter of the present invention can be introduced into a suitable restriction position in the vector. The contained plasmid is used for the transformation in *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium and then harvested and lysed. The plasmid is then recovered. As a method of analysis there is generally used sequence analysis, restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted and connected with the-next DNA sequence. Each sequence can be cloned in the same or different plasmid. After each introduction method of the desired promoter or construct or nucleotide sequence according to the present invention in the plants the presence and/or insertion of further DNA sequences may be necessary. If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblaserdam, 1985. Chapter V; Fraley, et al., Crit. Rev. Plant Sci., 4:1-46; and An et al., EMBO J. (1985) 4:277-284.

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. et al. (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

With this technique, infection of a plant may be done on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the promoter and/or the GOI, a plant to be infected is wounded, e.g. by cutting the plant with a razor or puncturing the plant with a needle or rubbing the plant with an abrasive. The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then
5 grown on a suitable culture medium and allowed to develop into mature plants.

When plant cells are constructed, these cells may be grown and maintained in accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant
10 hormones, vitamins, etc. Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue cultures, for example by selecting transformed shoots using an antibiotic and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

15

Further teachings on plant transformation may be found in EP-A-0449375.

In summation, the present invention provides a glucanase enzyme and the nucleotide sequence coding for the same. In addition, it provides a promoter that can control the
20 expression of that, or another, nucleotide sequence. In addition it includes terminator and signal sequences for the same.

The following sample was deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited
25 (NCIMB) at 23 St. Machar Drive, Aberdeen, Scotland, United Kingdom, AB2 1RY on 16 January 1995:

E. coli containing plasmid pEGLA-3 {i.e. *E. coli* DH5 α -pEGLA-3}. The deposit
number is NCIMB 40704.

30

The present invention will now be described by way of example.

In the following Examples reference is made to the accompanying Figures in which

Figures 1-10 are sequences of promoters and GOIs of earlier patent applications that are useful for use with the aspects of the present invention;

5

Figure 11 is a plasmid map of plasmid pEGLA-3;

Figure 12 is a schematic diagram of some promoter deletions;

10 Figure 13 is a plasmid map of pGPAMY;

Figure 14 is a graph;

Figure 15 is a plasmid map of pGP-GssAMY-Hyg;

15

Figure 16 is a graph; and

Figure 17 is a Western Blot.

20 The following Examples discuss recombinant DNA techniques. General teachings of recombinant DNA techniques may be found in Sambrook, J., Fritsch, E. F., Maniatis T. (Editors) Molecular Cloning. A laboratory manual. Second edition. Cold Spring Harbour Laboratory Press. New York 1989.

25 **Purification of the β -glucanase**

Aspergillus niger 3M43 was grown in medium containing wheat bran and beet pulp. The fermentation broth was separated from the solid part of the broth by filtration. Concentrated fermentation broth was then loaded on a 25X100mm Q-SEPHAROSE (Pharmacia) high Performance column, equilibrated with 20 mM Tris. HCl pH 7.5, and a linear gradient from 0-500 mM NaCl was performed and fractions of the eluate was collected. The β -glucanase eluted at ca 100 mM NaCl. The fractions containing

30

glucanase were combined and desalted using a 50x200 mm G-25 SEPHAROSE Superfine (Pharmacia). The column was then eluted with distilled water. After desalting the enzyme was concentrated using High-Trap spin columns.

5 Next the concentrated and desalted fractions were subjected to gel filtration on a 50x600 mm SUPERDEX 50 column. The sample was loaded and the column was eluted with 0.2 M Phosphate buffer pH 7.0 plus 0.2 M NaCl, and fractions of the eluate were collected. The fractions containing glucanase were combined and desalted and concentrated as described above.

10

The combined fractions were loaded on a 16X100 mm PhenylSEPHAROSE High Performance column (Pharmacia), equilibrated with 50 mM Phosphate buffer pH 6.0, containing 1.5 M $(\text{NH}_4)_2\text{SO}_4$. A gradient where the $(\text{NH}_4)_2\text{SO}_4$ concentration was varied from 1.5 - 0 M was applied and the eluate collected in fractions. The fractions
15 containing glucanase were combined. The purity of the β -1,4-glucanase was evaluated SDS-PAGE using the Phast system gel (Pharmacia).

Characterization

20 The molecular weight of the purified glucanase was determined by mass spectrometry using laser desorption technology. The MW of the glucanase was found to be 24,235 D \pm 50 D.

The pI value was determined by use of a Broad pI Kit (Pharmacia). The glucanase has
25 a pI value of about 4.

After SDS-PAGE analysis, treatment PAS reagent showed that the glucanase is not glycosylated. The PAS staining was done according to the procedure of I. Van-Seuningen and M. Davril (1992) Electrophoresis 13 pp 97-99.

30

Amino acid sequencing of the β -glucanase

The enzyme was digested with endoproteinase Lys-C sequencing grade from Boehringer Mannheim using a modification of the method described by Stone & Williams 1993 (Stone, K.L. and Williams, K.R. (1993). Enzymatic digestion of Proteins and HPLC Peptide Isolation. In : Matsudaira P. (Editor). A practical Guide to Protein and Peptide Purification for Microsequencing. Second Edition. Academic Press, San Diego 1993. pp 45-73).

Freeze dried β -glucanase (0.4 mg) was dissolved in 50 μ l of 8M urea, 0.4 M NH_4HCO_3 , pH 8.4. After overlay with N_2 and addition of 5 μ l of 45 mM DTT, the protein was denatured and reduced for 15 min at 50°C under N_2 . After cooling to RT, 5 μ l of 100 mM iodoacetamide was added for the cysteines to be derivatised for 15 min at RT in the dark under N_2 . Subsequently, 90 μ l of water and 5 μ g of endoproteinase Lys-C in 50 μ l of 50 mM Tricine and 10 mM EDTA, pH 8.0, was added and the digestion was carried out for 24h at 37°C under N_2 . The resulting peptides were separated by reversed phase HPLC on a VYDAC C18 column (0.46 x 15 cm; 10 μ m; The Separations Group; California) using solvent A: 0.1% TFA in water and solvent B: 0.1% TFA in acetonitrile. Selected peptides were rechromatographed on a Develosil C18 column (0.46 x 10 cm; 3 μ m) using the same solvent system prior to sequencing on an Applied Biosystems 476A sequencer using pulsed-liquid fast cycles.

The following peptide sequences were found:

SEQ I.D. No. 4
SEQ I.D. No. 5
SEQ I.D. No. 6
SEQ I.D. No. 7
SEQ I.D. No. 8

Isolation of a PCR clone of a fragment of the gene

PCR primers were synthesised using an Applied Biosystems DNA synthesiser model 392. In this regard, PCR primers were synthesized from two of the found peptide sequences.
5 WEVWYGT from Seq I.D. No. 4 and WTWSGG from Seq I.D. No. 7. The primer derived from WEVWYGT (reversed) is shown as Seq I.D. No. 9 and the primer derived from WTWSGG is shown as Seq I.D. No. 10 - see below:

SEQ. I.D. No. 10

10 TGG ACN TGG WSN GGN GG
17 mer 256 mixture

SEQ. I.D. No. 9

CTN CCR TAC CAN ACY TCC CA
15 20 mer 64 mixture

PCR amplification was performed with 100 pmol of each of these primers in 100 μ l reactions using the Amplitaq II kit (Perkin Elmer). The program was:

20	<u>STEP</u>	<u>TEMP</u>	<u>TIME</u>
	1	94°C	2 min
	2	94°C	1 min
	3	55°C	2 min
	4	72°C	2 min
25	5	72°C	5 min
	6	5°C	SOAK

Steps 2-4 were repeated for 40 cycles.

30 The program was run on a PERKIN ELMER DNA Thermal Cycler.

A 350 bp amplified fragment was isolated and cloned into a pT7-Blue T-vector according to the manufacturer's instructions (Novagen). A fragment was isolated and sequenced. The found sequence showed that it was indeed a part of the glucanase gene.

5 Isolation of *A. niger* genomic DNA

1g. of frozen *A. niger* mycelium was ground in a mortar under liquid nitrogen. Following evaporation of the nitrogen cover, the ground mycelium was extracted with 15ml of an extraction buffer (100mM Tris·HCl, pH 8.0, 0.50mM EDTA, 500mM NaCl, 10mM
10 β-mercaptoethanol) containing 1ml 20% sodium dodecyl sulphate. After incubation at 65°C for 10 min. 5ml 5M KAc, pH 5.0, was added and the mixture further incubated, after mixing, on ice for 20 mins. The mixture was then centrifuged for 20 mins. and the supernatant mixed with 0.6 vol. isopropanol to precipitate the extracted DNA. After further centrifugation for 15 mins. the DNA pellet was dissolved in 0.7 ml TE (10mM
15 Tris, HCl pH 8.0, 1mM EDTA) and precipitated with 75 µl 3M NaAc, pH 4.8, and 500 µl isopropanol. After centrifugation the pellet was washed with 70% ETOH and dried under vacuum. The DNA was dissolved in 200 µl TE and stored at -20°C.

Construction of a library

20

20 µg genomic DNA was partly digested with Tsp509I, which gives ends which are compatible with EcoRI ends. The digested DNA was separated on a 1 % agarose gel and fragments of 4-10 kb was purified. A λZAPII EcoRI/CIAP kit from Stratagene was used for library construction according to the manufacturers instructions. 2 µl of the ligation
25 (totally 5 µl) was packed with Gigapack Gold II packing extract according to the manufacturer's instructions (Stratagene). The library contained 650.000 independent clones.

Screening of the library

2 X 50.000 pfu was plated on NZY plates (5g NaCl, 2mg MgSO₄.7H₂O, 5g yeast extract, 10g casein hydrolysate, 15 g agar per liter) and plaquelifts were done on Hybond N sheets (Amersham). The sheets were hybridized with the PCR clone labelled with ³²P dCTP (Amersham) using Ready-to-go labelling kit from Pharmacia. The plaquelifts and hybridization were done in duplicate and positive clones were reckoned only when hybridization could be detected on both sheets. The nucleotide sequence of the present invention was sequenced using a ALF-laser fluorescence sequencer (Pharmacia). The sequence contained all the found amino acid sequence, confirming that the isolated gene indeed encoded the β -1,4-endoglucanase.

Sequence information

SEQ. ID. No. 12 presents the promoter sequence, the enzyme coding sequence, the terminator sequence and the signal sequence and the amino acid sequence of the enzyme of the present invention.

Testing enzyme activity

The purified protein was assayed for endo β -1,4 glucanase activity using Azurine-crosslinked barley β -glucan tablet (trade name: Glucazyme tablets supplied by Megazyme, Australia) by the instructions given by the manufacturer.

The purified enzyme gave a high activity on this substrate. Typically the enzyme has a specific activity of 2250 micromol glucose per min per mg of protein.

Induction of the Eg1A gene: identification of inducing carbon source

The Table below shows the identification of a number of high and low molecular weight inducers of the glucanase promoter. This analysis was carried out using the full length glucanase promoter of the present invention fused to the *E coli* β -glucuronidase gene.

The inducing strength of different carbon sources was determined quantitatively by measuring the intracellular GUS specific activity to hydrolyse p-nitrophenol glucuronide.

	CARBON SOURCE	GUS ACTIVITY
5	<u>(1%)</u>	<u>(units/mg)- 24 hours</u>
	xylose	12.91
	xylitol	10.62
	arabinose	8.50
10	arabitol	14.40
	glucose	20.25
	cellubiose	19.44
	xylo-oligomer 70	11.80
	glucopyranoside	19.70
15	methyl-xylopyranoside	12.60
	xyloglucan	13.90
	pectin	9.70
	arabinogalactan	30.20
	arabitol + glucose	29.50

20

Surprisingly glucose, which is normally a potent catabolite repressor, induces the glucanase promoter.

Accordingly, the present invention also relates to a promoter that is inducible by glucose.

25

In addition, the present invention relates to the use of glucose to induce a promoter.

30

These aspects of the present invention are different to the teachings of WO 94/04673 which discloses a fungal promoter that is active in the presence of glucose. In this regard, the promoter of the present invention is not only active in the presence of glucose but that it is also inducible by glucose.

One of the advantages of having a glucanase promoter that is inducible by glucose is that the promoter can be used to express a GOI, and thereby be used to prepare a POI (such as an heterologous POI), in a glucose containing environment. This is important because glucose is one of preferred carbon sources for biomass accumulation. In addition, glucose containing media are expected to produce lower amounts of proteases, thereby providing better yields of the POI. In addition, the use of a derepressed promoter in a derepressed host strain will increase the speed and efficiency of reaction media, such as a fermentation reaction medium. In addition, the use of mixed carbon sources during fermentation will allow the efficient induction of this promoter, for example at low levels of glucose and a cheap carbon source (e.g. sugar beet pulp).

Effects of promoter deletions on the regulation of the expression of the glucanase gene

A series of deletion studies, which are shown in Figure 12, were performed. In these studies, the different promoter deletion constructs shown in Figure 12 were fused to the GUS gene. The activity of the reporter gene was assayed qualitatively. The results showed that none of the deletions abolished the inducibility of the glucanase promoter. These results indicate the presence of multiple sites for transcriptional activation and initiation of transcription.

HETEROLOGOUS PROTEIN PRODUCTION USING TRANSFORMANTS OF ASPERGILLUS NIGER COMPRISING THE GLUCANASE PROMOTER (GP) AND THE GLUCANASE SIGNAL SEQUENCE (Gss)

Transformation of *Aspergillus Niger*

The protocol for transformation of *A. niger* was based on the teachings of Buxton, F.P., Gwynne D.I., Davis, R.W. 1985 (Transformation of *Aspergillus niger* using the *argB* gene of *Aspergillus nidulans*. Gene 37:207-214), Daboussi, M.J., Djeballi, A., Gerlinger, C., Blaiseau, P.L., Cassan, M., Lebrun, M.H., Parisot, D., Brygoo, Y. 1989 (Transformation of seven species of filamentous fungi using the nitrate reductase gene of

Aspergillus nidulans. Curr. Genet. 15:453-456) and Punt, P.J., van den Hondel, C.A.M.J.J. 1992 (Transformation of filamentous fungi based on hygromycin B and Phleomycin resistance markers. Meth. Enzym. 216:447-457).

5 For the purification of protoplasts, spores from one PDA (Potato Dextrose Agar - from Difco Lab. Detroit) plate of fresh sporulated N400 (CBS 120.49, Centraalbureau voor Schimmelcultures, Baarn) (7 days old) are washed off in 5-10 ml water. A shake flask with 200 ml Potato Dextrose Broth (difco 0549-17-9, Difco Lab. Detroit) is inoculated with this spore suspension and shaken (250 rpm) for 16-20 hours at 30°C.

10

The mycelium is harvested using Miracloth paper and 3-4 g wet mycelium are transferred to a sterile petri dish with 10 ml STC (1.2 M sorbitol, 10 mM Tris HCl pH 7.5, 50 mM CaCl_2) with 75 mg lysing enzymes (Sigma L-2265) and 4500 units lyticase (Sigma L-8012).

15

The mycelium is incubated with the enzyme until the mycelium is degraded and the protoplasts are released. The degraded mycelium is then filtered through a sterile 60 μm mesh filter. The protoplasts are harvested by centrifugation 10 min at 2000 rpm in a swing out rotor. The supernatant is discarded and the pellet is dissolved in 8 ml 1.5 M MgSO_4 and then centrifuged at 3000 rpm for 10 min.

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The upper band, containing the protoplasts is transferred to another tube, using a transfer pipette and 2 ml 0.6 M KCl is added. Carefully 5 ml 30% sucrose is added on the top and the tube is centrifuged 15 min at 3000 rpm.

25

The protoplasts, lying in the interface band, are transferred to a new tube and diluted with 1 vol. STC. The solution is centrifuged 10 min at 3000 rpm. The pellet is washed twice with STC, and finally solubilized in 1 ml STC. The protoplasts are counted and eventually concentrated before transformation.

30

For the transformation, 100 μ l protoplast solution (10^6 - 10^7 protoplasts) are mixed with 10 μ l DNA solution containing 5- 10 μ g DNA and incubated 25 min at room temperature. Then 60 % PEG-4000 is carefully added in portions of 200 μ l, 200 μ l and 800 μ l. The mixture is incubated 20 min at room temperature. 3 ml STC is added to the mixture and carefully mixed. The mixture is centrifugated 3000 rpm for 10 min.

The supernatant is removed and the protoplasts are solubilized in the remaining of the supernatant. 3-5 ml topagarose is added and the protoplasts are quickly spread on selective plates.

10

Glucanase promoter and heterologous gene expression

Figure 13 shows the expression vector pGPAmy that was used in these studies. This expression vector comprises the glucanase promoter fused to the *Thermomyces lanuginosus* precursor form of the α -amylase gene. Transcription from the promoter is terminated using the xylanase A terminator. This construct was used in a co-transformation experiment with the hygromycin resistance gene as the selectable marker.

The production of α -amylase using four independent transformants containing the expression vector pGPAmy when grown on sugar beet pulp and wheat bran is shown in Figure 14. The α -amylase activity was first detected in the culture medium after 48 hours of growth. A peak of enzyme activity was observed after days 3 and 4.

Glucanase signal sequence & heterologous protein production

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For these studies, the expression vector pGPGssAmyHyg was used.

The vector pGPGssAmyHyg is shown in Figure 15. This vector comprises the mature α -amylase gene translationally fused to the glucanase signal peptide (labelled ss). In addition, this vector comprises the promoter of the present invention (labelled EG1 A) and the xylanase A terminator. Transcription from this vector is therefore under the control of the glucanase promoter and termination by the xylanase A terminator.

30

This construct was used to test *inter alia* the efficiency of the signal peptide in heterologous protein secretion.

Figure 16 shows the results of the induction of α -amylase by use of the construct in strain 6M179 when grown in sugar beet pulp/wheat bran. The results show that the enzyme activity was localised in the culture medium and was first detected after 48 hours of growth. Accumulation of enzyme activity was observed at day 4.

Western Blot

10

Figure 17 shows a Western blot of proteins from the supernatant of three independent transformants separated by SDS-PAGE and blotted to a membrane. A synthetic peptide with 15 amino acid residues of *T lanuginosus* α -amylase recognised a single band on Western blots of culture supernatants from the transformants.

15

Antibody Production

Antibodies were raised against the enzyme of the present invention by injecting rabbits with the purified enzyme and isolating the immunoglobulins from antiserum according to procedures described according to N Harboe and A Ingild ("Immunization, Isolation of Immunoglobulins. Estimation of Antibody Titre" In A Manual of Quantitative Immuno-electrophoresis. Methods and Applications, N H Axelsen, *et al* (eds.), Universitetsforlaget, Oslo, 1973) and by T G Cooper ("The Tools of Biochemistry", John Wiley & Sons, New York, 1977).

25

SUMMARY

Even though it is known that *Aspergillus niger* produces several enzymes which can degrade β -glucan, the present invention provides a novel and inventive β -1,4-endoglucanase, as well as the coding sequence therefor, the termination sequence therefor, the signal sequence therefor, and the promoter for those sequences. An important advantage of the present invention is that the enzyme can be produced in high

30

amounts. In addition, the promoter and the regulatory sequences (such as the signal sequence and the terminator) can be used to express or can be used in the expression of GOIs in organisms, such as in *A. niger*.

- 5 The enzyme of the present invention is advantageous for feed supplements. In addition, it can be used in the brewing industry as it has a high fibre-conversion potential. In addition, there are fewer processing problems when the enzyme is used, particularly with non-starchy polysaccharides. In addition, the enzyme efficiently degrades β -glucans, therefore it can be used advantageously in the brewing industry to lower viscosity and
10 also improve the filterability of beer. This is important as large molecular weight glucans in beer and the like can cause filtration difficulties and give rise to sediments, gels and hazes.

- The signal sequence of the present invention is useful for secretion of a POI, such as a
15 heterologous POI, thereby improving the quality and quantity of the POI.

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the invention.

SEQUENCE INFORMATION

ENZYME SEQUENCE

SEQ ID NO: 1:

Gln	Thr	Met	Cys	Ser	Gln	Tyr	Asp	Ser	Ala	Ser	Ser	Pro	Pro	Tyr	Ser
1				5					10					15	
Val	Asn	Gln	Asn	Leu	Trp	Gly	Glu	Tyr	Gln	Gly	Thr	Gly	Ser	Gln	Cys
			20					25					30		
Val	Tyr	Val	Asp	Lys	Leu	Ser	Ser	Ser	Gly	Ala	Ser	Trp	His	Thr	Lys
		35					40					45			
Trp	Thr	Trp	Ser	Gly	Gly	Glu	Gly	Thr	Val	Lys	Ser	Tyr	Ser	Asn	Ser
	50					55					60				
Gly	Leu	Thr	Phe	Asp	Lys	Lys	Leu	Val	Ser	Asp	Val	Ser	Ser	Ile	Pro
65					70					75				80	
Thr	Ser	Val	Thr	Trp	Ser	Gln	Asp	Asp	Thr	Asn	Val	Gln	Ala	Asp	Val
			85					90						95	
Ser	Tyr	Asp	Leu	Phe	Thr	Ala	Ala	Asn	Ala	Asp	His	Ala	Thr	Ser	Ser
			100					105					110		
Gly	Asp	Tyr	Glu	Leu	Met	Ile	Trp	Leu	Ala	Arg	Tyr	Gly	Ser	Val	Gln
	115						120					125			
Pro	Ile	Gly	Lys	Gln	Ile	Ala	Thr	Ala	Thr	Val	Gly	Gly	Lys	Ser	Trp
	130					135					140				
Glu	Val	Trp	Tyr	Gly	Thr	Ser	Thr	Gln	Ala	Gly	Ala	Glu	Gln	Lys	Thr
145					150					155				160	
Tyr	Ser	Phe	Val	Ala	Gly	Ser	Pro	Ile	Asn	Ser	Trp	Ser	Gly	Asp	Ile
			165						170				175		
Lys	Asp	Phe	Phe	Asn	Tyr	Leu	Thr	Gln	Asn	Gln	Gly	Phe	Pro	Ala	Ser
		180						185					190		
Ser	Gln	His	Leu	Ile	Thr	Leu	Gln	Phe	Gly	Thr	Glu	Pro	Phe	Thr	Gly
	195						200					205			
Gly	Pro	Ala	Thr	Phe	Thr	Val	Asp	Asn	Trp	Thr	Ala	Ser	Val	Asn	*
	210						215					220			

ENZYME CODING SEQUENCE

SEQ ID NO: 2:

CAG ACG ATG TGC TCT CAG TAT GAC AGT GCC TCG AGC CCC CCA TAC TCG
GTG AAC CAG AAC CTC TGG GGC GAA TAC CAG GGC ACT GGC AGC CAG TGT
GTC TAC GTC GAC AAG CTT AGC AGC AGT GGT GCC TCA TGG CAT ACC AAA
TGG ACC TGG AGT GGT GGC GAG GGA ACA GTG AAA AGC TAC TCT AAC TCC
GGC CTT ACG TTT GAC AAG AAG CTA GTC AGC GAT GTG TCA AGC ATT CCC
ACC TCG GTG ACA TGG AGC CAG GAC GAC ACC AAT GTC CAA GCC GAT GTC
TCA TAT GAT CTG TTC ACC GCG GCG AAT GCG GAT CAT GCC ACT TCC AGC
GGT GAC TAT GAG CTT ATG ATT TGG CTT GCC CGC TAC GGC TCA GTC CAG
CCT ATT GGC AAG CAG ATT GCC ACG GCC ACT GTG GGA GGC AAG TCC TGG
GAG GTG TGG TAT GGT ACC AGC ACC CAG GCC GGT GCG GAG CAA AAG ACA
TAT AGC TTC GTG GCA GGA TCT CCT ATC AAC TCG TGG AGT GGG GAC ATT
AAG GAC TTC TTC AAC TAT CTC ACC CAG AAC CAA GGC TTC CCG GCT AGC
TCT CAG CAT TTG ATC ACT CTG CAA TTT GGA ACT GAG CCG TTC ACC GGT
GGC CCG GCA ACC TTC ACG GTT GAC AAC TGG ACC GCT AGT GTC AAC

PROMOTER SEQUENCE

SEQ ID NO: 3:

AATTGAAGCA	TTTTGATAGG	TTTAAGCCTA	ATCAGGATAT	TGGATGAGTC	GAGTTGCAGA	60
AGTTGAGGAC	GGTGGGTGAA	ATCGGGGGTT	TGATAGGTAG	GCAATGCAGG	GCGGAACGGG	120
AAGGGTCTAG	ACAATTTCTT	TCTTTTGGAC	AGCTGGTGCG	TTTCACTGAG	ATTAATAGTA	180
AGCAAACACTAC	TCGCTCGAAG	TCGTAGATGT	GCATAATGGA	TAACACAGC	CAACCGAAAT	240
CTCCGGGCAG	AAGGCCTGGA	GGCAGGAGGA	AACGTGGATA	AGAGAGTAAT	GTTTGAGTAT	300
AGATATGTAG	GCAAGAAAGG	ACTGGGAGGA	AGGAAGTATC	GCAAACAAGA	CAAGTCACTG	360
AATAGGAAAG	AATGGGGCCA	TCAGAGAAAT	GAATCTAAAC	GGTAACTGCA	GATATTACAT	420
GGAAGAAAAT	ACTATGATCC	CTAATTGATA	TGGTTCCATG	GCCCCTGGAG	ACTTAAACCT	480
CGTGGTATGA	TAAACATATG	AGTTACATTC	TCGGTAAATC	CAACATTACT	CCCAAGCTCT	540
GTTGATATTC	TCCGATAATT	CACCGATAAC	CAACCAACCT	ACTCCCGTCT	AGATCCAATT	600
GGTCTATATG	CATAATGGAT	ATCGTCAGCA	CAGGCAGAAC	CCTTTAATTT	ATTTCTGGAG	660
ATCCCGTTCT	CCACAATGCT	TGGTTGCCGA	CTGCCACAGA	CCATCGCTAA	CTTGAAGCGG	720
AAAGTGCTCC	GATGAAGGGT	CTCATTTTGA	AACGGAGGAT	TTACATGTCA	ATGTTGCAGG	780
CTGGCGTTGA	TGATGGCGCA	ACCTGCTATA	GCTAGTTGGC	TTACTTCGTC	CTGGCTGCCG	840
TATTGGACAC	GGAAAGTCGG	ACAATAATAG	TGTTAACAGT	AAGCGCCATT	GATCAGAGTT	900
GATGTATTTA	AAGCTGCGTC	GTCTGCTGCC	CCCTCCGTGT	TCGTGTCTTA	TTCCAAACAT	960
TCAACCTCTA	TTCTTTTCGA	AGTCCTTTAG	ATCTGCCGTT	CCTCTGCTTT	ATTGCCCAAC	1020

INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Aspergillus niger*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser Trp Glu Val Trp Tyr Gly Thr Ser Thr Gln Ala Gly Ala Glu Gln

1

5

10

15

Lys

INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Thr Tyr Ser Phe Val Ala Gly Ser Pro Ile

1

5

10

INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

43

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Lys	Leu	Val	Ser	Asp	Val	Ser	Ser	Ile	Pro	Thr	Ser	Val	Thr	Xaa	Ser
1					5				10				15		
Gln	Asp	Asp	Thr	Asn	Xaa	Xaa	Ala	Ala	Val	Ser	Tyr	Xaa	Leu	Phe	Thr
			20					25					30		
Ala	Ala	Asn													
			35												

INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Trp	Thr	Trp	Ser	Gly	Gly	Glu	Gly	Thr	Val	Lys
1			5					10		

INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Leu	Ser	Ser	Ser	Gly	Ala	Ser	Trp	His	Thr	Lys
1				5				10		

INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide"

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GTN CCR TAC CAN ACY TCC CA 17

INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide"

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TGG ACN TGG WSN GGN GG 17

INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 345 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "PCR fragment"

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTGGAGTGGT GGCAGGGGAA CAGTGAAAAG CTA CTCTAAC TCGGSCCTTA CTTTGACAA	60
GAAGCTAGTC AGCGATGTGT CAAGCATTCC CACCTCGGTG ACATGGAGCC AGGACGACAC	120
CAATGTCCAA GCCGATGTCT CATATGATCT GTTCACCGCG GCGAATGCGG ATCATGCCAC	180
TTCCAGCGGT GACTATGAGC TTATGATTG STATGTGACG TCGTGAACAA GATAGATGGA	240
GGAGGCTAAC GTAACCAGGC TTGCCCGCTA CGGCTCAGTC CAGCCTATTG GCAAGCAGAT	300
TGCCACGGCC ACTGTGGGAG GCAAGTCCTG GGAGGTCTGG TACGG	345

INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2360 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Aspergillus niger*
- (B) STRAIN: 3M43

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(1021..1427, 1476..1708, 1778..1857)
- (D) OTHER INFORMATION: /product= "Endoglucanase"
/gene= "eglA"

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 1021..1427

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 1428..1475

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 1476..1708

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 1709..1777

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 1778..1854

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 1021..1068

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: join(1069..1427, 1476..1708, 1777..1854)

(ix) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AATTGAAGCA TTTTGATAGG TTTAAGCCTA ATCAGGATAT TGGATGAGTC GAGTTGCAGA	60
AGTTGAGGAC GGTGGGTGAA ATCGGGGGTT TGATAGGTAG GCAATGCAGG GCGGAACGGG	120
AAGGGTCTAG ACAATTTCTT TCTTTTGGAC AGCTGGTGCG TTTCAGTGAG ATTAATAGTA	180
AGCAAACCTAC TCGCTCGAAG TCGTAGATGT GCATAATGGA TAACTACAGC CAACCGAAAT	240
CTCCGGGCAG AAGGCCTGGA GGCAGGAGGA AACGTGGATA AGAGAGTAAT GTTTGAGTAT	300
AGATATGTAG GCAAGAAAGG ACTGGGAGGA AGGAAGTATC GCAAACAAGA CAAGTCACTG	360
AATAGGAAAG AATGGGGCCA TCAGAGAAAT GAATCTAAAC GGTAAGTGCA GATATTACAT	420
GGAAGAAAAT ACTATGATCC CTAATTGATA TGGTTCCATG GCCCCTGGAG ACTTAAACCT	480
CGTGGTATGA TAAACATATG AGTTACATTC TCGGTAAATC CAACATTACT CCCAAGCTCT	540
GTTGATATTC TCGGATAATT CACCGATAAC CAACCAACCT ACTCCCGTCT AGATCCAATT	600
GGTCTATATG CATAATGGAT ATCGTCAGCA CAGGCAGAAC CCTTTAATTT ATTTCTGGAG	660
ATCCCGTTCT CCACAATGCT TGGTTGCCGA CTGCCACAGA CCATCGCTAA CTGAAGCGG	720
AAAGTGCTCC GATGAAGGGT CTCATTTTGA AACGGAGGAT TTACATGTCA ATGTTGCAGG	780
CTGGCGTTGA TGATGGCGCA ACCTGCTATA GCTAGTTGGC TTACTTCGTC CTGGCTGCCG	840
TATTGGACAC GGAAAGTCGG ACAATAATAG TGTTAACAGT AAGCGCCATT GATCAGAGTT	900
GATGTATTTA AAGCTGCGTC GTCTGCTGCC CCCTCCGTGT TCGTGTCTTA TTCCAAACAT	960
TCAACCTCTA TTCCTTTTGA AGTCCTTTAG ATCTGCCGTT CCTCTGCTTT ATTGCCCAAC	1020
ATG AAG CTC TCC ATG ACA CTT TCC CTG TTT GCG GCC ACT GCC ATG GGC	1068
Met Lys Leu Ser Met Thr Leu Ser Leu Phe Ala Ala Thr Ala Met Gly	
-16 -15 -10 -5	
CAG ACG ATG TGC TCT CAG TAT GAC AGT GCC TCG AGC CCC CCA TAC TCG	1116
Gln Thr Met Cys Ser Gln Tyr Asp Ser Ala Ser Ser Pro Pro Tyr Ser	
1 5 10 15	
GTG AAC CAG AAC CTC TGG GGC GAA TAC CAG GGC ACT GGC AGC CAG TGT	1164
Val Asn Gln Asn Leu Trp Gly Glu Tyr Gln Gly Thr Gly Ser Gln Cys	
20 25 30	
GTC TAC GTC GAC AAG CTT AGC AGC AGT GGT GCC TCA TGG CAT ACC AAA	1212
Val Tyr Val Asp Lys Leu Ser Ser Ser Gly Ala Ser Trp His Thr Lys	
35 40 45	
TGG ACC TGG AGT GGT GGC GAG GGA ACA GTG AAA AGC TAC TCT AAC TCC	1260
Trp Thr Trp Ser Gly Gly Glu Gly Thr Val Lys Ser Tyr Ser Asn Ser	
50 55 60	
GGC CTT ACG TTT GAC AAG AAG CTA GTC AGC GAT GTG TCA AGC ATT CCC	1308
Gly Leu Thr Phe Asp Lys Lys Leu Val Ser Asp Val Ser Ser Ile Pro	
65 70 75 80	

ACC TCG GTG ACA TGG AGC CAG GAC GAC ACC AAT GTC CAA GCC GAT GTC 1356
 Thr Ser Val Thr Trp Ser Gln Asp Asp Thr Asn Val Gln Ala Asp Val
 85 90 95
 TCA TAT GAT CTG TTC ACC GCG GCG AAT GCG GAT CAT GCC ACT TCC AGC 1404
 Ser Tyr Asp Leu Phe Thr Ala Ala Asn Ala Asp His Ala Thr Ser Ser
 100 105 110
 GGT GAC TAT GAG CTT ATG ATT TG GTATGTGACG TCGTGAACAA 1447
 Gly Asp Tyr Glu Leu Met Ile Trp
 115 120
 GATAGATGGA GGAGGCTAAC GTAACCAG G CTT GCC CGC TAC GGC TCA GTC CAG 1500
 Leu Ala Arg Tyr Gly Ser Val Gln
 125
 CCT ATT GGC AAG CAG ATT GCC ACG GCC ACT GTG GGA GGC AAG TCC TGG 1548
 Pro Ile Gly Lys Gln Ile Ala Thr Ala Thr Val Gly Gly Lys Ser Trp
 130 135 140
 GAG GTG TGG TAT GGT ACC AGC ACC CAG GCC GGT GCG GAG CAA AAG ACA 1596
 Glu Val Trp Tyr Gly Thr Ser Thr Gln Ala Gly Ala Glu Gln Lys Thr
 145 150 155 160
 TAT AGC TTC GTG GCA GGA TCT CCT ATC AAC TCG TGG AGT GGG GAC ATT 1644
 Tyr Ser Phe Val Ala Gly Ser Pro Ile Asn Ser Trp Ser Gly Asp Ile
 165 170 175
 AAG GAC TTC TTC AAC TAT CTC ACC CAG AAC CAA GGC TTC CCG GCT AGC 1692
 Lys Asp Phe Phe Asn Tyr Leu Thr Gln Asn Gln Gly Phe Pro Ala Ser
 180 185 190
 TCT CAG CAT TTG ATC A GTGAGTTTTT CTAATTCTAC TAGCGAGCGC 1738
 Ser Gln His Leu Ile
 195
 CGGCAGTTGA AATTGGTCAC TAACAGAAGT GATGATTAG CT CTG CAA TTT GGA 1791
 Thr Leu Gln Phe Gly
 200
 ACT GAG CCG TTC ACC GGT GGC CCG GCA ACC TTC ACG GTT GAC AAC TGG 1839
 Thr Glu Pro Phe Thr Gly Gly Pro Ala Thr Phe Thr Val Asp Asn Trp
 205 210 215
 ACC GCT AGT GTC AAC TAA AAGGCTTTAG GCGCGGCTGG GGTAAATAAC 1887
 Thr Ala Ser Val Asn *
 220
 AGCTTGTTTC TTGTTCTAG AACGTGGGGC GTGTAAGAGC TAGAAATCCA CCCACTGTGA 1947

TTGGAAACAC TCATTCAAGA TCGGTACTCC TCTTCAGCCG AGAAAGGCAC AGATAGTGTA	2007
TCGAATCCAA TCAAATCTAT TTGGTGTTGC TTAAATTCCG AGCCAGTCCT TTCCTTGAAA	2067
GGTAATCCAC CCGTAGCGAT TGATCATTAA CAGATCCGAG TGGTGCTAGG TTAAATTGCT	2127
AACCCGATCC CGCTCCAATT AGCTAGCGCA TCCGGCAGAT TCAAAC TTGA CAGTGGGCCG	2187
GGCATTACCT GAACCTGTAG AAGGAACAGA CCCTTGCTA GAAATCTCTA AATAGTATAA	2247
GCCGAAACTT GCCCGGACG TACCCTAAGC TAAGATTGCT CTTCGCATTC CCAGGGGGGT	2307
GAACTCTCTA AAGAGGGAGC ATCGCTTGCC GATGTCTGGT TCGGGGATCA TGA	2360

(2) INFORMATION FOR SEQ ID NO: 13.

TERMINATOR SEQUENCE

AAGGCTTTAG GCGCGGCTGG GGTAATAAC AGCTTGTTTC TTCGTTCTAG	50
AACGTCGGGC GTGTAAGAGC TAGAAATCCA CCCACTCTGA TTGGAAACAC	100
TCATTCAAGA TCGGTACTCC TCTTCAGCCG AGAAAGGCAC AGATAGTGTA	150
TCGAATCCAA TCAAATCTAT TTGGTGTTGC TTAAATTCCG AGCCAGTCCT	200
TTCCTTGAAA GGTAATCCAC CCGTAGCGAT TGATCATTAA CAGATCCGAG	250
TGGTGCTAGG TTAAATTGCT AACCCGATCC CGCTCCAATT AGCTAGCGCA	300
TCCGGCAGAT TCAAAC TTGA CAGTGGGCCG GGCATTACCT GAACCTGTAG	350
AAGGAACAGA CCCTTGCTA GAAATCTCTA AATAGTATAA GCCGAAACTT	400
GCCCCGACG TACCCTAAGC TAAGATTGCT CTTCGCATTC CCAGGGGGGT	450
GAACTCTCTA AAGAGGGAGC ATCGCTTGCC GATGTCTGGT TCGGGGATCA	500
TGA	5037

(2) INFORMATION FOR SEQ ID NO: 14:

SIGNAL SEQUENCE

ATG AAG CTC TCC ATG ACA CTT TCC CTG TTT GCG GCC ACT GCC ATG GGC	48
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(2) INFORMATION FOR SEQ ID NO: 15:

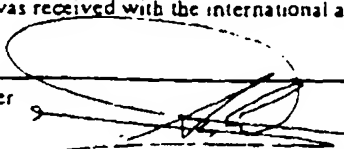
SIGNAL SEQUENCE

Met Lys Leu Ser Met Thr Leu Ser Leu Phe Ala Ala Thr Ala Met Gly	16
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>26</u> , line <u>28 and 29</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution The National Collections of Industrial and Marine Bacteria Limited (NCIMB)	
Address of depositary institution (including postal code and country) 23 St. Machar Drive Aberdeen Scotland AB2 1RY United Kingdom	
Date of deposit <u>16 JANUARY 1995</u>	Accession Number <u>NCIMB 40704</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
<p>In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).</p>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	
<input checked="" type="checkbox"/> This sheet was received with the international application	
Authorized officer  J. van Aubel	

For International Bureau use only	
<input type="checkbox"/> This sheet was received by the International Bureau on:	
Authorized officer	

CLAIMS

1. An enzyme obtainable from *Aspergillus*, wherein the enzyme has the following characteristics:
 - a. a MW of $24,235 \text{ D} \pm 50 \text{ D}$
 - b. a pI value of about 4
 - c. glucanase activity
- wherein the glucanase activity is endo β -1,4-glucanase activity.
2. An enzyme having sequence shown as SEQ. I.D. No. 1 or a variant, homologue or fragment thereof.
3. An enzyme coded by the nucleotide sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.
4. A nucleotide sequence coding for the enzyme according to claim 1.
5. A nucleotide sequence coding for the enzyme according to claim 2.
6. A nucleotide sequence having the sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.
7. A nucleotide sequence according to any one of claims 4 to 6 operatively linked to a promoter.
8. A nucleotide sequence according to claim 7 wherein the promoter is the promoter having the sequence shown as SEQ. I.D. No. 3 or a variant, homologue or fragment thereof or a sequence complementary thereto.

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9. A promoter having the sequence shown as SEQ. I.D. No. 3 or a variant, homologue or fragment thereof or a sequence complementary thereto.
10. A promoter according to claim 9 operatively linked to a GOI.
- 5 11. A promoter according to claim 10 wherein the promoter is operatively linked to a GOI, wherein the GOI comprises a nucleotide sequence according to any one of claims 4-6.
- 10 12. A terminator having the nucleotide sequence shown as SEQ. I.D. No. 13 or a variant, homologue or fragment thereof or a sequence complementary thereto.
13. A signal sequence having the nucleotide sequence shown as SEQ. I.D. No. 14 or a variant, homologue or fragment thereof or a sequence complementary thereto.
- 15 14. A construct comprising or expressing the invention according to any one of claims 1 to 13.
15. A vector comprising or expressing the invention of any one of claims 1 to 14.
- 20 16. A plasmid comprising or expressing the invention of any one of claims 1 to 15.
17. A transgenic organism comprising or expressing the invention according to any one of claims 1 to 16.
- 25 18. A transgenic organism according to claim 17 wherein the organism is a fungus.
19. A transgenic organism according to claim 17 wherein the organism is a filamentous fungus, preferably *Aspergillus*.
- 30 20. A transgenic organism according to claim 17 wherein the organism is a plant.

21. A transgenic organism according to claim 17 wherein the organism is a yeast.
22. A process of preparing an enzyme according to any one of claims 1 to 3 comprising expressing a nucleotide sequence according to any one of claims 4-8.
- 5 23. A process according to claim 22 wherein the enzyme has the sequence shown as SEQ. I.D. No. 1 or a variant, homologue or fragment thereof, and the nucleotide sequence has the sequence shown as SEQ. I.D. No. 2 or a variant, homologue or fragment thereof or a sequence complementary thereto.
- 10 24. A process according to claim 22 or claim 23 wherein the expression is controlled (partially or completely) by use of a promoter according to claim 9.
- 15 25. A process for expressing a GOI by use of a promoter, wherein the promoter is the promoter according to claim 9.
26. Use of an enzyme according to any one of claims 1 to 3 or prepared by a process according to any one of claims 22 to 25 to degrade a glucan.
- 20 27. Plasmid NCIMB 40704, or a nucleotide sequence obtainable therefrom for expressing a glucanase enzyme or for controlling the expression thereof or for controlling the expression of another GOI.
- 25 28. A glucanase enzyme having the ability to degrade β -1,4-glucosidic bonds, which is immunologically reactive with an antibody raised against a purified glucanase enzyme having the sequence shown as SEQ. I.D. No. 1.
29. A signal sequence having the sequence shown as SEQ. I.D. No. 15 or a variant, homologue or fragment thereof.

FIGURE 1

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AMY 637 PROMOTER

SEQUENCE TYPE: Nucleotide

MOLECULE TYPE: DNA

ORIGINAL SOURCE: *Solanum Tuberosum*

SEQUENCE LENGTH: 2094

SEQUENCE:

10	20	30	40
ATTAAGGGGA	GCATAAGTGC	AGCTCAGAAA	TTCACACCTG
50	60	70	80
ATATTTTCCC	AAAGCCCTCA	AAAATGTGAA	CAAATCTGCT
90	100	110	120
AAAATGTCAG	TCAGAAGGAC	TGTTCTTTTA	GGTTTTCTTC
130	140	150	160
TCTCGAGTCA	CGAAATCAGA	TAATATGATA	AGAAATTATG
170	180	190	200
GAGGATTTAT	AATGTATCTG	TCTGTTCTTA	GGTATAATTA
210	220	230	240
TGTGTTCCCT	TATGATGTAG	TAATGGAATT	CTGGGCTTAT
250	260	270	280
ATTAAAGGAA	CTGAATATAA	ATGTTGCGAT	TTTAACTGCG
290	300	310	320
GAGACTTCGA	GTTAGAGCCT	TATAATTATG	TCTTATCATT
330	340	350	360
TTATACTGAG	ATCATATTAC	AGATGATGAA	AGCTGACATT
370	380	390	400
GCATTAGTTA	TTCTGTTTTA	TACAAGTCAT	GTAAGTCTG
410	420	430	440
CTTGTGAGTT	GTGACTGTAA	GATAAATTGA	TTCAGCCTTC
450	460	470	480
TGTGGCATT	GCGGAGATCT	GATTATACTC	TCATCGTCTT
490	500	510	520
ATCTAAGTTG	CTCATGCAAC	TTTGTCCCTG	ATAGTTGGCT
530	540	550	560
AATACTACAA	CTGGAATTAA	GTGTAGTTAT	TCGAAATCTC
570	580	590	600
TGTTGGAAGT	TGCTAAGTGC	TTAAGTGCTG	GTTATTGTAA
610	620	630	640
ACCCCATCCG	AGTTATTATA	CAGCATCTGG	CTGATGAAAT
650	660	670	680
GCTGCTCATT	TGCAATGGTG	ACATAACCAA	ATGTTAGTAA
690	700	710	720
AACATACTAG	CTGGTTGAAT	GTTAGATGAT	TGTTCAACGT
730	740	750	760
TACATCTCAC	AGAAACCTTA	TTATGGATTG	ACATGTTAGT
770	780	790	800
TGATCCGAAA	GATCCTTCTT	TTAAATGCCA	AAGCTTGTTA
810	820	830	840
CAGATTTGAG	GAGTTCTTTT	ACTTCTTTTT	GTTATATCTA
850	860	870	880
TTTCCCATTC	ATTTTGACGT	TCAGCCTCAC	AGATGTTGTC
890	900	910	920
ATACTTAGAA	ATGTGCGTAT	ATATATAGAG	AGAGAGAGAT
930	940	950	960
AGAGTGAAAT	GATTATATAG	TCSAAGATTA	CGAAACTTGA

970	980	990	1000
CATTGAGACA	TCTGTGATTG	TTTGAAATTT	ATGTATATAT
1010	1020	1030	1040
CTGTAGCATT	AGAAACTATA	AGAGTTGTTA	GCTTCACTTG
1050	1060	1070	1080
TCTTATTGTT	GTGCTCAAAG	CAACTTCATC	ATACAGTATG
1090	1100	1110	1120
GTTTTTATAT	GCTCTCCAT	TATCACCGAA	CCTTATGATT
1130	1140	1150	1160
ATGTGTACGA	GCTTATAATA	TTACTGATGG	TGATTGAGTA
1170	1180	1190	1200
TTATGATTAT	GTCTCCATT	AATTATTCTG	TTTCATACAA
1210	1220	1230	1240
GTCGTGTAAT	TTGCTGTTTG	TGATTGTACG	ATAAATTGAT
1250	1260	1270	1280
TCAACCTTCT	GCGGTGTTGG	TTGAAGTTCA	AGTAAATTAG
1290	1300	1310	1320
CTTTATTTAT	CATAGTAGCA	TTTGATTATT	GATGCTCTGT
1330	1340	1350	1360
AGCTAATGAT	AAGCCATTGA	AGGGAAGCAG	AAATGGTAAA
1370	1380	1390	1400
GCTTTCCTAAA	ATGAATCTAC	GAATGGATGA	TAAAGTTAAT
1410	1420	1430	1440
GAATATTGTT	GATACTTCTG	CAATCAGATT	ATGAGTTACT
1450	1460	1470	1480
GAGTCTACTG	TTTTTTAAGC	CTGTTTCAGA	TGATCGATCA
1490	1500	1510	1520
TCAACAACAA	CATATTCAGT	GTAGTAGACA	TGATCGATCA
1530	1540	1550	1560
CTTTCTAATT	TTGATTATG	CACCCCTCTT	TCTCCAATTT
1570	1580	1590	1600
GGTCGTCTTC	TTTTTTTCAT	GATGTCACTG	AATTATTCTC
1610	1620	1630	1640
TGGTCGTCCC	CACCATTCAG	GAAGTCACTT	CGAGCATAAT
1650	1660	1670	1680
GTGAAAACAT	CCACATTTTT	CAAATCCAGC	AGAATTTTCA
1690	1700	1710	1720
TCAAACGGG	TTCAACATTT	ACTACATGTA	TACACTCTGA
1730	1740	1750	1760
AGTCTGAATC	CACTAATTCT	AGATGGTGCA	TCTGTGCCCC
1770	1780	1790	1800
CACACTTGTG	AAAGCTTATT	CTCAATTTTT	TATTTTCCAA
1810	1820	1830	1840
CAACTTGAAT	TCAGACCACA	CAACTCCCGT	GTCTTGACG
1850	1860	1870	1880
GTCAGCATCT	GAGTGGAGAA	CTCAATTAAG	TGACTTTAAC
1890	1900	1910	1920
GTCGAGTTCT	ATAGTAAACA	ACCCCTATAT	CTTTTTTCAA
1930	1940	1950	1960
GCAATGTAAG	ATTGCGAACA	CACTGAAATT	TCCAGGTGGT
1970	1980	1990	2000
TAATCTTGTA	CCCAGTGTGT	GTACTTTTAA	AAAAAAAAGT
2010	2020	2030	2040
CAGTTTATA	GTCTCTAAAA	CACATTTTAA	TAGAGTTTAT
2050	2060	2070	2080
TTGCCATCTT	TTGTTCTCTA	TACTAGACTT	CGGAGTCAAC
2090			
ACAACACAAC	AACA		

FIGURE 2

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AMY 351 PROMOTER

SEQUENCE TYPE: Nucleotide

MOLECULE TYPE: DNA (genomic)

ORIGINAL SOURCE: *Solanum tuberosum*

SEQUENCE LENGTH: 1734 bp

STRANDEDNESS: Double

TOPOLOGY: Linear

SEQUENCE:

10	20	30	40
TCITTAAGTT	GTTTGCTTGA	TTTTTCTTCT	TCAATCTTCT
50	60	70	80
ATATTTAATT	CGTTTTAGCT	TCAAACCTTCT	TCAATTTTAT
90	100	110	120
TTCAATTTAA	TTCTACAAAA	AAAATCTCTA	TTTAGCACCA
130	140	150	160
TTCATAAAAAT	TCATGCTCAA	AATGGGCAAA	CATAAATAAT
170	180	190	200
AAATGTGAAG	TAAATAATGG	ATTAAAATAT	ATATTTTTGG
210	220	230	240
GCCTCACATC	AACCTTCATA	ATTCTTGAAT	GAATGAATGA
250	260	270	280
TAGACTTCAT	AATTTTTTAA	CCTATACATA	TAAGAAAATT
290	300	310	320
GAGAGTAACT	CAAATAACAA	GTTGTAGTAT	CACATCTTTA
330	340	350	360
CTATTTGATA	ACATTATGAA	GGTGATTATA	CATTACGTAA
370	380	390	400
CATTTCTTTT	AAAAATATGT	AAGCAAATTT	ACTTTTTAAC
410	420	430	440
TTATCATTGA	TCTTCATGGT	TTTGTCTATA	ATCTCAAAGT
450	460	470	480
TATCATATTT	TATATAGCTA	TTTGAAAGTA	ATTTTATTTT
490	500	510	520
TACTCATCAT	TGAGTGATGC	TTTTATTATA	ATACTAGTAA
530	540	550	560
GTTTTATTTA	TTATTTTCTT	TTAGGGGTGA	ATTGTATAAT
570	580	590	600
ATAATAAAAA	ATATATTTT	AGAAATAATG	ATTCTTTTAT
610	620	630	640
TATTA AAAAG	TTAAGATATT	AGATTATTTA	TGCTTGATA
650	660	670	680
ATAATGAACG	AAGTTTTATT	TTCTATGAGT	TTCAATTAATC
690	700	710	720
ATGTTTGTA	TTATTTCAAA	TTTTGATGTA	TTTTTATAAT
730	740	750	760
TTTGTATTAT	TATATTATTA	TACTATATTT	AAAAATTATA
770	780	790	800
AGATCCATAG	GGCTTACGCC	CCACGTCAAG	AGGCTTGCGC
810	820	830	840
CTTTCCTTAA	ATTAAGTAAA	ACTCTTCGCC	TCATGCCTTA
850	860	870	880
CGCCTCCGCC	TTTTAAAACA	CTGATTCCTT	TCCTCATATA
890	900	910	920
GCTTGAGGCG	AAAATATTTA	ATAAAAACAC	TTCTTAATTT
930	940	950	960
GTTTATATGT	TCAATTGAAC	ATGTCCGTGA	TTAGAAAATT

FIGURE 2 CONTINUED

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970	980	990	1000
AAATTAAATT	CAATGACAAA	TTTAATAATT	TGACACAAAA
1010	1020	1030	1040
TTTATGAAAA	AAATATCAAA	ATATAAAGAA	ATATTTTTTT
1050	1060	1070	1080
TGAAATGGAT	TAAAAAGAAA	AAAAAAACAA	ATAAATTGAA
1090	1100	1110	1120
CCGGGATAAG	TTGGTTGTTT	AATTGATTAT	TGATTATGAT
1130	1140	1150	1160
CTCAATTTGA	CATTTTGCGC	GATCTTTTGA	CCTCAATTCC
1170	1180	1190	1200
TATGAAGTGA	CACTACGCCA	ATGGACAGTC	GCCGTCGTCA
1210	1220	1230	1240
CCGCCACCGC	ACTATTCTCG	ACGCGTCGTC	TATCTCCTCC
1250	1260	1270	1280
ACCCACACAGC	CGTCAATTCC	AAGCTTCCAA	TGAACCGTTG
1290	1300	1310	1320
CCATGTGTCA	CTGCCTATTC	ACCGCGAAAC	ATGAATATCA
1330	1340	1350	1360
CTGACGAACG	ATTTGCGAGC	GGAACGAATC	CAGAAAATGG
1370	1380	1390	1400
ATTACTTTCT	ATAAAATCCT	CGAATCTCAA	CTCCATTTCT
1410	1420	1430	1440
TAAAAATAAA	ATTAAAAATA	TTGTTTCTTT	TTGTATTCTT
1450	1460	1470	1480
TTTTGTATTT	CTGGTTTATG	TGGTGATCGA	ATTTTCAATT
1490	1500	1510	1520
TTTTTACTGG	TAGTGATTCC	TACTTTTCTT	CAATTGCATT
1530	1540	1550	1560
TCTCCTTTTT	CCATTTACAG	GTTGAGAATT	CATGATTCTT
1570	1580	1590	1600
TATCAGAGGA	ATCGATCCGA	TTTGACTAAT	TTCACTTTTT
1610	1620	1630	1640
GTCTGTATAA	ATACCAGAGT	ATCTAGGTTG	AGGAACGTAA
1650	1660	1670	1680
TTTCAAGCTG	CGATCGGCTT	TTTCCCCTGA	ACGAGCAAAC
1690	1700	1710	1720
ACAGGTTGTG	GGTTCGAGTT	AGCAAGGGAC	GTATAATCTC
1730			
AACTACAATC	CATT		

FIGURE 3

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α -AMYLASE CODING SEQUENCE

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2017 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (A) LENGTH: 475 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

ATG AAG TCT CTC GCC GCA ATT GCT GCT CTG CTG TCG CCC ACA CTG GTC	48
Met Lys Ser Leu Ala Ala Ile Ala Ala Leu Leu Ser Pro Thr Leu Val	
-18 -15 -10 -5	
CGG GCA GCG ACT CCG GAT GAG TGG AAA GCT CAG TCG ATC TAT TTC ATG	96
Arg Ala Ala Thr Pro Asp Glu Trp Lys Ala Gln Ser Ile Tyr Phe Met	
1 5 10	
CTG ACG GAC CGG TTT GCG CGT ACC GAC AAT TCG ACC ACG GCT CCC TGT	144
Leu Thr Asp Arg Phe Ala Arg Thr Asp Asn Ser Thr Thr Ala Pro Cys	
15 20 25 30	
GAC ACC ACT GCC GGG GTATGCAACT AACCTGTGT TTCTCTTCCC GGGACGTACA	199
Asp Thr Thr Ala Gly	
35	
AGGGGTCTTC TCCATGCTAA CCGTGACAT GCAG AAA TAT TGC GGG GGA ACA	251
Lys Tyr Cys Gly Gly Thr	
40	
TGG CGA GGT ATC ATC AAC AAC GTAAGTGGCT TCTGATTTTC GCTCAATAAT	302
Trp Arg Gly Ile Ile Asn Asn	
45	
CTTCGTCGCG TGACTTTATT TCCTAG CTG GAT TAC ATC CAG GAT ATG GGC TTC	355
Leu Asp Tyr Ile Gln Asp Met Gly Phe	
50 55	
ACA GCT ATC TGG ATA ACT CCA GTG ACA GCC CAG TGG GAC GAC GAT GTG	403
uThr Ala Ile Trp Ile Thr Pro Val Thr Ala Gln Trp Asp Asp Asp Val	
60 65 70	
GAT GCG GCA GAT GCA ACG TCG TAT CAC GGT TAT TGG CAG AAA GAC CT	450
Asp Ala Ala Asp Ala Thr Ser Tyr His Gly Tyr Trp Gln Lys Asp Leu	
75 80 85	
GTGCGCAACC CTGCTCCATG GATCGCTGGC TGCAAACTCG TGCTGATCGG TGATTTT	510
TTTTTTTTT TGGAAACAG A TAC TCT CTG AAT TCG AAA TTC GGC ACT GCC	560
Tyr Ser Leu Asn Ser Lys Phe Gly Thr Ala	
90 95	

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FIGURE 3 CONTINUED

GAT GAC TTG AAA GCC CTG GCT GAT GCC CTT CAC GCC CGT GGG ATG CTT Asp Asp Leu Lys Ala Leu Ala Asp Ala Leu His Ala Arg Gly Met Leu 100 105 110 115	508
CTC ATG GTC GAC GTC GTG GCT AAT CAC TTT GTACGGACCA TCTACATACC Leu Met Val Asp Val Val Ala Asn His Phe 120 125	658
TGGGAAACGC GAAGAAGGAA AAAAAAAGGCGCACGC TAACATTTCG CGTTTAG	715
GGC TAC GGC GGT TCT CAT AGC GAG GTG GAT TAC TCG ATC TTC AAT CCT Gly Tyr Gly Gly Ser His Ser Glu Val Asp Tyr Ser Ile Phe Asn Pro 130 135 140	763
CTG AAC AGC CAG GAT TAC TTC CAC CCG TTC TGT CTC ATT GAG GAC TAC Leu Asn Ser Gln Asp Tyr Phe His Pro Phe Cys Leu Ile Glu Asp Tyr 145 150 155	811
GAC AAC CAG GAA GAA GTC GAA CAA TGC TGG CTG GCC GAT ACT CCG ACG Asp Asn Gln Glu Glu Val Glu Gln Cys Trp Leu Ala Asp Thr Pro Thr 160 165 170	859
ACA TTG CCC GAC GTG GAC ACC ACC AAT CCT CAG GTT CCG ACG TTT TTC Thr Leu Pro Asp Val Asp Thr Thr Asn Pro Gln Val Arg Thr Phe Phe 175 180 185	907
AAC GAC TGG ATC AAG AGC CTG GTG GCG AAC TAC TCC A GTATGATTGT Asn Asp Trp Ile Lys Ser Leu Val Ala Asn Tyr Ser 190 195 200	954
TCCCGCGGTA ACGCTTTAGG GCTTGCTCTA ACTGAAATCG ACAG TC GAT GGT CTG Ile Asp Gly Leu 205	1009
CGC GTC GAC ACC GTT AAG CAC GTG GAG AAA GAT TTC TGG CCC GAC TTC Arg Val Asp Thr Val Lys His Val Glu Lys Asp Phe Trp Pro Asp Phe 210 215 220	1057
AAC GAA GCT GCT GCG TGT ACC GTC GGC GAG GTG TTC AAC GGT GAC CCA Asn Glu Ala Ala Ala Cys Thr Val Gly Glu Val Phe Asn Gly Asp Pro 225 230 235	1105
GCG TAC ACC TGC CCA TAC CAG GAA GTG CTG GAT GGC GTT CTG AAC TAT Ala Tyr Thr Cys Pro Tyr Gln Glu Val Leu Asp Gly Val Leu Asn Tyr 240 245 250	1153
CCG AT GTGAGTGATT CCGAAAGTTC CATCGATCAG GCTTCTGAC GCATGAGAAC Pro Ile 255	1208

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FIGURE 3 CONTINUED

AGC TAC TAT CCT GCG CTT GAT GCA TTC AAG TCT GTC GGC GGC AAT CTC	1256
Tyr Tyr Pro Ala Leu Asp Ala Phe Lys Ser Val Gly Gly Asn Leu	
260 265 270	
GGC GGC TTG GCT CAG GCC ATC ACC ACC GTG CAG GAG AGC TGC AAG GAT	1304
Gly Gly Leu Ala Gln Ala Ile Thr Thr Val Gln Glu Ser Cys Lys Asp	
275 280 285	
TCC AAT CTG CTC GGC AAT TTC CTT GAG AAT CAC GAC ATT GCT CGC TTT	1352
Ser Asn Leu Leu Gly Asn Phe Leu Glu Asn His Asp Ile Ala Arg Phe	
290 295 300	
GCT TC GTATGGACAC TCTTTTGTAA GCCCTCATCG ATTGGGGATG CTGACACGGA	1407
Ala Ser	
CAACAACAAC AG G TAC ACG GAT GAC CTT GCT CTC GCC AAG AAT GGT CTC	1456
Tyr Thr Asp Asp Leu Ala Leu Ala Lys Asn Gly Leu	
305 310 315	
GCT TTC ATC ATC CTC TCG GAT GGT ATT CCG ATC ATC TAC ACG GGC CAG	1504
Ala Phe Ile Ile Leu Ser Asp Gly Ile Pro Ile Ile Tyr Thr Gly Gln	
320 325 330	
GAG CAG CAC TAC GCC GGT GAT CAC GAT CCC ACA AAT CGT GAG GCC GTC	1552
Glu Gln His Tyr Ala Gly Asp His Asp Pro Thr Asn Arg Glu Ala Val	
335 340 345	
TGG CTG TCT GGC TAC AAT ACC GAC GCC GAG CTG TAC CAG TTC ATC AAG	1600
Trp Leu Ser Gly Tyr Asn Thr Asp Ala Glu Leu Tyr Gln Phe Ile Lys	
350 355 360	
AAG GCC AAT GGC ATC CGC AAC TTG GCT ATC AGC CAG AAC CCG GAA TTC	1648
Lys Ala Asn Gly Ile Arg Asn Leu Ala Ile Ser Gln Asn Pro Glu Phe	
365 370 375 380	
ACC TCC TCC AAG GTGAGTACAA TAACAACTT TTCGAAAAAT TTTTCACCGG	1700
Thr Ser Ser Lys	
AGAAAACCTA AGATTCGGCT AACAAAACAA AAAAAAAAAA G ACC AAG GTC ATC	1753
Thr Lys Val Ile	
385	
TAC CAA GAC GAT TCG ACC CTT GCC ATT AAC CGG GGC GGC GTC GTT ACT	1801
Tyr Gln Asp Asp Ser Thr Leu Ala Ile Asn Arg Gly Gly Val Val Thr	
390 395 400	
GTC CTG AGC AAT GAA GGC GCC TCC GGG GAG ACC GGG ACT GTC TCC ATT	1849
Val Leu Ser Asn Glu Gly Ala Ser Gly Glu Thr Gly Thr Val Ser Ile	
405 410 415 420	

FIGURE 3 CONTINUED

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CCG GGA ACT GGC TTC GAG GCC GGC ACG GAA TTG ACT GAT GTC ATC TCC	1897
Pro Gly Thr Gly Phe Glu Ala Gly Thr Glu Leu Thr Asp Val Ile Ser	
425 430 435	
TGC AAG ACC GTG ACT GCG GGG GAC AGC GGG GCG GTC GAC GTG CCC TTG	1945
Cys Lys Thr Val Thr Ala Gly Asp Ser Gly Ala Val Asp Val Pro Leu	
440 445 450	
TCG GGC GGA CTG CCA AGC GTG CTC TAT CCC AGC TCC CAG CTG GCC AAG	1993
Ser Gly Gly Leu Pro Ser Val Leu Tyr Pro Ser Ser Gln Leu Ala Lys	
455 460 465	
AGT GGT CTG TGT GCG TCG GCG TGA	2017
Ser Gly Leu Cys Ala Ser Ala	
470 475	

FIGURE 4

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α -AMYLASE CODING SEQUENCE
 SEQUENCE TYPE: Nucleotide
 MOLECULE TYPE: DNA
 ORIGINAL SOURCE: *Solanum Tuberosum*
 SEQUENCE LENGTH: 1570
 SEQUENCE:

10	20	30	40
TGTGGTGATC	GAATTTTCAA	TTTTTTTACT	GAGTATCTAG
50	60	70	80
GTTGAGGAAC	GTAATTTCAA	GCTGCGATCG	GCTTTTTCCC
90	100	110	120
CTGAACGAGC	AAACACAGGT	TGTGGGTTGG	AGTTAGCAAG
130	140	150	160
GGACGTATAA	TCTCAACTAC	AATCCATTAT	GGCGCTTGAT
170	180	190	200
GAAAGTCAGC	AGTCTGATCC	ATTGGTTGTG	ATACGCAATG
210	220	230	240
GAAAGGAGAT	CATATTGCAG	GCATTCGACT	GGGAATCTCA
250	260	270	280
TAAACATGAT	TGGTGGCTAA	ATTTAGATAC	GAAAGTTCCT
290	300	310	320
GATATTGCAA	AGTCTGGTTT	CACAACTGCT	TGGCTGCCTC
330	340	350	360
CGGTGTGTCA	GTCATTGGCT	CCTGAAGGTT	ACCTTCCACA
370	380	390	400
GAACCTTTAT	TCTCTCAATT	CTAAATATGG	TTCTGAGGAT
410	420	430	440
CTCTTAAAAG	CTTTACTTAA	TAAGATGAAG	CAGTACAAAG
450	460	470	480
TTAGAGCGAT	GGCGGACATA	GTCATTAACC	ACCGTGTTGG
490	500	510	520
GACTACTCAA	GGGCATGGTG	GAATGTACAA	CCGCTATGAT
530	540	550	560
GGAATTCCTA	TGTCTTGGGA	TGAACATGCT	ATTACATCTT
570	580	590	600
GCACTGGTGG	AAGGGGTAAC	AAAAGCACTG	GAGACAACCT
610	620	630	640
TAATGGAGTT	CCAAATATAG	ATCATACACA	ATCCTTTGTT
650	660	670	680
CGGAAAGATC	TCATTGACTG	GATGCGGTGG	CTAAGATCCT
690	700	710	720
CTGTTGGCTT	CCAAGATTTT	CGTTTTGATT	TTGCCAAAGG
730	740	750	760
TTATGCTTCA	AAGTATGTAA	AGGAATATAT	CGAGGGAGCT
770	780	790	800
GAGCCAATAT	TTGCAGTTGG	AGAATACTGG	GACACTTGCA
810	820	830	840
ATTACAAGGG	CAGCAATTTG	GATTACAACC	AAGATAGTCA
850	860	870	880
CAGGCAAAGA	ATCATCAATT	GGATTGATGG	CGCGGGACAA
890	900	910	920
CTTTCAACTG	CATTCGATTT	TACAACAAAA	GCAGTCCTTC

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FIGURE 4 CONTINUED

930	940	950	960
AGGAAGCAGT	CAAAGGAGAA	TTCTGGCGTT	TGGGTGACTC
970	980	990	1000
TAAGGGGAAG	CCCCCAGGAG	TTTTAGGATT	GTGGCCTTCA
1010	1020	1030	1040
AGGGCTGTCA	CTTTTATTGA	TAATCACGAC	ACTGGATCAA
1050	1060	1070	1080
CTCAGGCGCA	TTGGCCTTTC	CCTTCACGTC	ATGTTATGGA
1090	1100	1110	1120
GGGCTATGCA	TACATTCTTA	CACACCCAGG	GATACCATCA
1130	1140	1150	1160
GTTTTCTTTG	ACCATTCTTA	CGAATGGGAT	AATTCCATGC
1170	1180	1190	1200
ATGACCAAAT	TGTAAAGCTG	ATTGCTATTC	GGAGGAATCA
1210	1220	1230	1240
AGGCATACAC	AGCCGTTTCA	CTATAAGAAT	TCTTGAGGCA
1250	1260	1270	1280
CAGCCAAACT	TATACGCTGC	AACCATTGAT	GAAAAGGTTA
1290	1300	1310	1320
GCGTGAAGAT	TGGGGACGGA	TCATGGAGCC	CTGCTGGGAA
1330	1340	1350	1360
AGAGTGGACT	CTCGCGACCA	GTGGCCATCG	CTATGCAGTC
1370	1380	1390	1400
TGGCAGAAGT	AATCTTACAG	CTATTCCGTT	ACTTAATATA
1410	1420	1430	1440
TTAGTAGAAA	TATATATGTT	TTAAACCCGA	GCACCTACTT
1450	1460	1470	1480
CTAACACTAG	ATCCGCCTCT	ACAGGCTTGG	ATGGAGTGAT
1490	1500	1510	1520
GAGTTTTTTT	TTCCTGTTCA	TTAGACATTG	CAACATGGGA
1530	1540	1550	1560
TGTATGTTTT	GTTAATAAAA	GTGTTCTTGA	TCAATGCAAT
1570			
GTAATAAGGG			

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FIGURE 5

SEQUENCE: Nucleotide sequence of a cDNA encoding the large subunit of ADP-glucose pyrophosphorylase from barley seed endosperm (bep110)

SEQUENCE TYPE: NUCLEIC ACID

MOLECULE TYPE: DNA

ORIGINAL SOURCE: BARLEY

SEQUENCE LENGTH: 2037

STRANDEDNESS: DOUBLE

TOPOLOGY: LINEAR

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1   ACGACCACCT CCGAACTCAA CGCCTCCACG GACCATCTCT
41  CTCCTCTCCC CTCCCCTCAC CACCACCACC ACCACCACCC
81  CTTCTCCCTC CCTGCATTTG ATTCGTTTAT ATTTCATCCG
121 CGCTTGCCCG GTCGCCACCC CGTCGATCCC TCACCCCGCC
161 GTCCCCGGCA GTTGCAAGTG GACTGCTAAT GTCATCGATG
201 CAGTTCAGCA GCGTGCTGCC CCTGGAGGGC AAGGCGTGCG
241 TTTCCCAAGT CAGGAGAGAG GGATCGGCCT GCGAGCGCCT
281 CAAGATCGGG GACAGCAGCA GCATCAGGCA CGAGAGAGCG
321 TCCAGGAGGA TGTGCAACGG CGGCGCAGGG GCCCCGCCGC
361 CACCGGTGCG CAGTGCGTGC TCACCTCCGA CGCCAGCCCC
401 GCCGACACCC TTGTTCTCCG GACGTCTTTC CGGAGGAATT
    ACGCCGATCC GAACGAGGTC GCGGCCGTGC GTCGCGGCCG
    TCATACTCGG CGGCGGCACC GGGACTCAGC TCTTCCCCT
    CACAAGCACA AGGGCCACAC CTGCTGTTCC TATTGGAGGA
    TGTTACAGGC TCATCGATAT TCCCATGAGC AACTGCTTCA
601 ACAGTGGCAT CAACAAGATA TTCGTCATGA CCCAGTTCAA
    CTCGGCATCT CTCAATCGCC ACATTCACCG CACCTACCTC
    GGCAGGGGAA TCAATTTTAC TGATGGATCT GTTGAGGTAT
    TGGCCGCGAC ACAAATGCCT GGGGAGGCTG CTGGATGGTT
    CCGCGGAACA GCGGATGCCG TCAGAAAATT TATCTGGGTG
801 CTTGAGGACT ACTATAAGCA TAAATCCATA GAGCACATTT
    TGATCTTGTC GGGCGATCAG CTTTATCGCA TGGATTACAT
    GGAGCTTGTC CAGAAACATG TGGATGACAA TGCTGACATT
    ACTTTATCAT GTGCCCCTGT TGGAGAGAGC CGGGCATCTG
    AGTACGGGCT AGTGAAGTTC GACAGTTCAG GCCGTGTGAT
1001 CCAGTTTTTCT GAGAAGCCAA AGGGCGACGA TCTGGAAGCG
    ATGAAAGTGG ATACCAGTTT TCTCAATTTT GCCATAGACG
    ACCCTGCTAA ATATCCATAC ATTGCTTCGA TGGGAGTTTA
    TGCTTTCAAG AGAGATGTTT TGCTGAACCT TCTAAAGTCA
    AGATACGCAG AACTACATGA CTTTGGGTCT GAAATCCTCC
1201 CGAGAGCTCT GCATGATCAC AATGTACAGG CATATGTCTT
    CACTGACTAC TGGGAGGACA TTGGAACAAT CAGATCCTTC
    TTCGATGCCA ACATGGCCCT CTGCGAACAG CCTCCAAAGT
    TTGAATTTTA TGATCCAAAA ACCCCCTTCT TCACTTCGCG
    TCGGTACTTA CCGCCAACAA AGTCAGACAA GTGCAGGATC
1401 AAAGAAGCGA TCATTTTCGA CGGCTGCTTC TTGCGTGAA
    GCAAAATCGA GCACTCCATC ATCGGCGTTC GTTCACGCC
    AAATCCGGGA AGCGAGCTCA AGAACGCGAT GATGATGGGC
    GCGGACTCGT ACGAGACCGA GGACGAGATC TCGAGGCTGA
    TGTCTGAGGG CAAGGTTCCC ATCGGCGTGC GGGAGAACAC
1601 AAAGATCAGC AACTGCATCA TCGACATGAA CGCGAGGATA

```

FIGURE 5 CONTINUED

	GGAAGGGACG	TGGTCATCTC	AAACAAGGAG	GGGGTGCAAG
	AAGCCGACAG	GCCGGAGGAA	GGGTACTACA	TCAGGTCCGG
	GATCGTGGTG	ATCCAGAAGA	ACGCGACCAT	CAAGGACGGC
	ACCGTCGTGT	AGGGCGTGCC	GGGTCGGCGC	GACGGGGTTC
1801	TGCGACAACC	TGTGCGCTGC	GTCGGTCGTC	ATCATCTTCT
	CAAAC TCCGG	GACTGAAGAA	GTGATCCGGG	GACGGGAGAC
	GTTTGAAGCT	TGAATGACTG	AGACTGAAAG	TGAAGGCCCA
	GCAGAGGCAG	GCAGCATTAG	TAGTAAGTAG	TAAGTAAGTA
	GCAGTGGAAC	AAAGTAATAG	TCGTTGTTTT	TTCCCTGTGA
2001	ATAAATAAGA	GGCTGTGTGT	TGAGGTAAAA	AAAAAAA

FIGURE 6

SEQUENCE: Nucleotide sequence of a cDNA encoding the small subunit of ADP-glucose pyrophosphorylase from barley seed endosperm (beps)

SEQUENCE TYPE: NUCLEIC ACID

MOLECULE TYPE: DNA

ORIGINAL SOURCE: BARLEY

SEQUENCE LENGTH: 1822

STRANDEDNESS: DOUBLE

TOPOLOGY: LINEAR

COMMENT: The "." at 1569 denotes a purine.

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1   AAAAGTGAAC TCACACATCA CTCAATATCT ATATCCTTCC
    ATTTTATATC CCTCGGTGAT GGATGTACCT TTGGCATCTA
    AAGTTCCCTT GCCCTCCCTT TCCAAGCATG AACAAATGCAA
    CGTTTATAGT CATAAGAGCT CATCGAAGCA TGCAGATCTC
    AATCCCCATG CTATTGATAG TGTTCCTCGGT ATCATTCTTG
201  GAGGTGGTGC AGGGACTAGA TTGTATCCCC TGACGAAGAA
    GCGTGCAAAG CCTGCAGTGC CATTGGGTGC CAACTACAGG
    CTTATTGATA TTCCTGTCAG TAATTGTCTG AACAGCAACA
    TATCAAAGAT CTATGTGCTT ACACAGTTCA ACTCAGCTTC
    TCTTAATCGT CATCTCTCAC GAGCCTATGG GAGCAACATT
401  GGAGGTTACA AGAATGAAGG ATTTGTTGAA GTCCTTGCTG
    CACAGCAGAG CCCAGATAAC CCTGACTGGT TCCAGGGTAC
    TGCAGATGCT GTAAGGCAGT ACTTGTGGCT ATTCGAGGAG
    CATAATGTTA TGGAGTATCT AATTCTTGCT GGAGATCACC
    TGTACCGAAT GGACTATGAA AAGTTTATTC AGGCACACAG
601  AGAAACGGAT GCTGATATTA CTGTTGCTGC CTTGCCCATG
    GATGAGGAAC GTGCAACTGC ATTTGGCCTT ATGAAAATCG
    ATGAAGAAGG GAGGATAATT GAATTCGCAG AGAAACCAAA
    AGGAGAACAG TTGAAAGCTA TGATGGTTGA TACGACCATA
    CTTGGCCTTG AAGATGCGAG GGCAAAGGAA ATGCCTTATA
801  TTGCTAGCAT GGGTATCTAT GTTATTAGCA AACATGTGAT
    GCTTCAGCTT CTCCGTGAGC AATTTCTTGG AGCTAATGAC
    TTCGGAAGTG AAGTTATCCC TGGTGCAACT AGCACTGGCA
    TGAGGGTACA AGCATACCTA TACGACGGTT ACTGGGAAGA
    TATTGGTACA ATTGAGGCAT TCTATAATGC AAATTTGGGA
1001 ATTACCAAAA AACCAATACC TGATTTAGT TTCTATGACC
    GTTCTGCTCC CATTTACACA CAACCTCGAC ACTTGCTCC
    TTCAAAGGTT CTTGATGCTG ATGTGACAGA CAGTGTAATT
    GGTGAAGGAT GTGTTATTAA AAAGTGAAG ATACACCATT
    CAGTAGTTGG ACTCCGTTCC TGCATATCTG AAGGTGCAAT
1201 AATAGAGGAC ACGTTGCTAA TGGGTGCGGA CTACTATGAG
    ACTGAAGCTG ATAAGAACT CCTTGCTGAA AAAGGTGGCA
    TTCCCATTTG TATTGGAAAG AATTCACACA TCAAAAGAGC
    AATCATTGAC AAGAATGCTC GTATTGGAGA TAACGTGATG
    ATAATCAATG TTGACAATGT TCAAGAAGCG GCGAGGGAGA
1401 CAGATGGATA CTTTATCAAA AGTGGCATCG TAACTGTGAT
    CAAGGATGCT TTAATCCCTA GTGGAACAGT CATATGAAGC
    AGATGTGAAA TGTATGCCAA AAGACAGGGC TACTTGCGTC
    AGTCTGGAAT CAACCAACAA GGCCGCGAAG GAGATCATAA
    AATAAAAAA G GAGTGCCATG CGAGTCACTT CTACACCTT
1601 TTCCCCCTT GATGTATTAG GAAGTGTGAT GTACAAGCAA
  
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FIGURE 6 CONTINUED

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CTGTGATGCA CTTACGCGAA GTGCCCTGG ATTCAGCTTT  
CTCTTTGCTT GTAAGTGGT TCCAGCAGAC CATGCTATTT  
GTTGTATGGT TCGTGCAAAA CCTTGCGATG CTTTATATAT  
GCTTTATATA TAAACAAGAT GAATCCCCGC GCGTTGCTGC  
2001 GGCACAAAAA AAAAAAAAAA AA
```

FIGURE 7

 α -GLUCAN LYASE CODING SEQUENCE

SEQUENCE TYPE: NUCLEIC ACID

MOLECULE TYPE: DNA (GENOMIC)

ORIGINAL SOURCE: FUNGALLY INFECTED ALGAE

SEQUENCE LENGTH: 3267 BP

STRANDEDNESS: DOUBLE

SEQUENCE:

	10	20	30	40	50	60
1	ATGTTTTCAA	CCCTTGCGTT	TGTGCGACCT	AGTGCGCTGG	GAGCCAGTAC	CTTCGTAGGG
61	GCGGAGGTCA	GGTCAAATGT	TGCTATCCAT	TCCGCTTTTC	CAGCTGTGCA	CACAGCTACT
121	CGCAAAACCA	ATCGCCTCAA	TGTATCCATG	ACCGCATTGT	CCGACAAACA	AACGGCTACT
181	GCGGGTAGTA	CAGACAATCC	GGACGGTATC	GACTACAAGA	CCTACGATTA	CGTGGGAGTA
241	TGGGGTTTCA	GCCCCCTCTC	CAACACGAAC	TGGTTTGCTG	CCGGCTCTTC	TACCCCGGGT
301	GGCATCACTG	ATTGGACGGC	TACAATGAAT	GTCAACTTCG	ACCGTATCGA	CAATCCGTCC
361	ATCACTGTCC	AGCATCCCGT	TCAGGTTTCT	GTCACGTCAT	ACAACAACAA	CAGCTACAGG
421	GTTTCGTTCA	ACCTGTATGG	CCCTATTTCG	GATGTGACTC	GTGGGCCTAT	CCTCAAGCAG
481	CAACTAGATT	GGATTCTAAC	GCAGGAGCTG	TCAGAGGGAT	GTGATCCCGG	AATGACTTTC
541	ACATCAGAAG	GTTTCTTGAC	TTTTGAGACC	AAGGATCTAA	GCGTCATCAT	CTACGGAAAT
601	TTCAAGACCA	GAGTTACGAG	AAAGTCTGAC	GGCAAGGTCA	TCATGGAAAA	TGATGAAGTT
661	GGAACGTGAT	CGTCCGGGAA	CAAGTGCCGG	GGATTGATGT	TCGTTGATAG	ATTATACGGT
721	AACGCTATCG	CTTCCGTCAA	CAAGAACTTC	CGCAACGACG	CGGTCAAGCA	GGAGGGATTTC
781	TATGGTGCAG	GTGAAGTCAA	CTGTAAGTAC	CAGGACACCT	ACATCTTAGA	ACGCACTGGA
841	ATCGCCATGA	CAAATTACAA	CTACGATAAC	TTGAACTATA	ACCAGTGGGA	CCTTAGACCT
901	CCGCATCATG	ATGGTGCCCT	CAACCCAGAC	TATTATATTC	CAATGTACTA	CGCAGCACCT
961	TGGTTGATCG	TTAATGGATG	CGCCGGTACT	TCGGAGCAGT	ACTCGTATGG	ATGGTTCATG
1021	GACAATGTCT	CTCAATCTTA	CATGAATACT	GGAGATACTA	CCTGGAATTC	TGGACAAGAG
1081	GACCTGGCAT	ACATGGGCGC	GCAGTATGGA	CCATTTGACC	AACATTTTGT	TTACGGTGCT
1141	GGGGGTGGGA	TGGAATGTGT	GGTCACAGCG	TTCTCTCTTC	TACAAGGCAA	GGAGTTCGAG
1201	AACCAAGTTC	TCAACAAACG	TTCAAGTAATG	CCTCCGAAAT	ACGTCTTTGG	TTTCTTCCAG
1261	GGTGTTCG	GGACTTCTTC	CTTGTGAGA	GCGCATATGC	CAGCAGGTGA	GAACAACATC
1321	TCAGTCAAG	AAATTGTAGA	AGGTTATCAA	AACAACAATT	TCCCTTTTCA	GGGGCTCGCT
1381	GTGGACGTGG	ATATGCAAGA	CAACTTGCGG	GTGTTACCA	CGAAGGGCGA	ATTTTGGACC
1441	GCAAACAGGG	TGGGTACTGG	CGGGGATCCA	AACAACCGAT	CGGTTTTTGA	ATGGGCACAT
1501	GACAAAGGCC	TTGTTTGTCA	GACAAATATA	ACTTGCTTCC	TGAGGAATGA	TAACGAGGGG
1561	CAAGACTACG	AGGTCAATCA	GACGTTAAGG	GAGAGGCAGT	TGTACACGAA	GAACGACTCC
1621	CTGACGGGTA	CGGATTTTGG	AATGACCGAC	GACGGCCCCA	GCGATGCGTA	CATCGGTTCAT
1681	CTGGACTATG	GGGGTGGAGT	AGAATGTGAT	GCACTTTTCC	CAGACTGGGG	ACGGCCTGAC
1741	GTGGCCGAAT	GGTGGGGAAA	TAACATAAAG	AAACTGTTCA	GCAATTGGTC	CGACTTCGTC
1801	TGGCAAGACA	TGACTGTTCC	AGCAATGATG	CCGCACAAAA	TTGGCGATGA	CATCAATGTG
1861	AAACCGGATG	GGAAATTGGC	GAATGCGGAC	GATCCGTCCA	ATGGACAATA	CAACTGGAAG
1921	ACGTACCATC	CCCAAGTGCT	TGTAACGTAT	ATGCGTTATG	AGAATCATGG	TGGGGAACCG
1981	ATGGTCACTC	AACGCAACAT	TCATGCGTAT	ACACTGTGCG	AGTCTACTAG	GAAGGAAGGG
2041	ATCGTGGAAG	ACGCAGACAC	TCTAACGAAG	TTCCGCCGTA	GCTACATTAT	CAGTCGTGGT
2101	GGTTACATTG	GTAACCAGCA	TTTCGGGGGT	ATGTGGGTGG	GAGACAACCT	TACTACATCA
2161	AACTACATCC	AAATGATGAT	TGCCAACAAT	ATTAACATGA	ATATGTCTTG	CTTGGCTCTC
2221	GTCCGGCTCCG	ACATTGGAGG	ATTCACTCA	TACGACAATG	AGAATCAGCG	AACGGCGTGT
2281	ACCGGGGACT	TGATGGTGAG	GTATGTGCAG	GCGGGCTGCC	TGTTGCCGTG	GTTGAGGAAC
2341	CACATATGATA	GGTGGATCGA	GTCCAAGGAC	CACGGAAAGG	ACTACCAGGA	GCTGTACATG
2401	TATCCGAATG	AAATGGATAC	GTTGAGGAAG	TTGCTTGAAT	TCCGTTATCG	CTGGCAGGAA
2461	GTGTTGTACA	CGGCCATGTA	CCAGAATGCG	GCTTTCGGAA	AGCCGATTAT	CAAGGCTGCT
2521	TCGATGTACA	ATAACGACTC	AAACGTTCCG	AGGGCGCAGA	ACGATCATTC	CGTTGTTGGT
2581	GGACATGATG	GATATCCCAT	TCTGTGCGCG	CCTGTTGTGT	GGGAGAAATC	GACCGAACCG

FIGURE 7 CONTINUED

2641	GAATTGTACT	TGCCCCGTGCT	GACCCAATGG	TACAAATTCG	GTCCCGACTT	TGACACCAAG
2701	CCTCTGGAAG	GAGCGATGAA	CGGAGGGGAC	CGAATTTACA	ACTACCCTGT	ACCGCAAGT
2761	GAATCACCAA	TCTTCGTGAG	AGAAGGTGCG	ATTCTCCCTA	CCCGCTACAC	GTTGAACGGT
2821	GAAAACAAAT	CATTGAACAC	GTACACGGAC	GAAGATCCGT	TGGTGTTTGA	AGTATTCCCC
2881	CTCGGAAACA	ACCGTGCCGA	CGGTATGTGT	TATCTTGATG	ATGGCGGTGT	GACCACCAAT
2941	GCTGAAGACA	ATGGCAAGTT	CTCTGTCGTC	AAGGTGGCAG	CGGAGCAGGA	TGGTGGTACG
3001	GAGACGATAA	CGTTTACGAA	TGATTGCTAT	GAGTACGTTT	TCGGTGGACC	GTTCTACGTT
3061	CGAGTGCGCG	GCGCTCAGTC	GCCGTGGAAC	ATCCACGTGT	CTTCTGGAGC	GGGTTCTCAG
3121	GACATGAAGG	TGAGCTCTGC	CACTTCCAGG	GCTGCGCTGT	TCAATGACGG	GGAGAACGGT
3181	GATTTCTGGG	TTGACCAGGA	GACAGATTCT	CTGTGGCTGA	AGTTGCCCAA	CGTTGTTCTC
3241	CCGGACGCTG	TGATCACAAAT	TACCTAA			

FIGURE 8

α -GLUCAN LYASE CODING SEQUENCE
 SEQUENCE TYPE: NUCLEIC ACID
 MOLECULE TYPE: DNA (GENOMIC)
 ORIGINAL SOURCE: FUNGALLY INFECTED ALGAE
 SEQUENCE LENGTH: 3276 BP
 STRANDEDNESS: DOUBLE
 SEQUENCE:

	10	20	30	40	50	60
1	ATGTATCCAA	CCCTCACCT	CGTGGCGCCT	AGTGGGCTAG	GGGCCAGAAC	TTTCACGTGT
61	GTGGGCATTT	TTAGGTCACA	CATTCTTATT	CATTGCGTTG	TTCCAGCGGT	GCGTCTAGCT
121	GTGGCGAAAA	GCAACCGCCT	CAATGTATCC	ATGTCCGCTT	TGTTGACAAA	ACCGACTGCT
181	GTTACTGGAG	GGAAGGACAA	CCCGGACAAT	ATCAATTACA	CCACTTATGA	CTACGTCCCT
241	GTGTGGCGCT	TCGACCCCT	CAGCAATACG	AACTGGTTTG	CTGCCGGATC	TTCCACTCCC
301	GGCGATATTG	ACGACTGGAC	GGCGACAATG	AATGTGAACT	TCGACCGTAT	CGACAATCCA
361	TCCTTCACTC	TCGAGAAACC	GGTTCAGGTT	CAGGTCACGT	CATACAAGAA	CAATTGTTTC
421	AGGGTTTCGT	TCAACCTGA	TGGTCCTATT	CGCGATGTGG	ATCGTGGGCC	TATCCTCCAG
481	CAGCAACTAA	ATTGGATCCG	GAAGCAGGAG	CAGTCGAAGG	GGTTTGATCC	TAAGATGGGC
541	TTCACAAAAG	AAGGTTTCTT	GAAATTTGAG	ACCAAGGATC	TGAACGTTAT	CATATATGGC
601	AATTTTAAGA	CTAGAGTTAC	GAGGAAGAGG	GATGGAAAAG	GGATCATGGA	GAATAATGAA
661	GTGCCGGCAG	GATCGTTAGG	GAACAAGTGC	CGGGGATTGA	TGTTTGTCGA	CAGGTTGTAC
721	GGCACTGCCA	TCGCTTCCGT	TAATGAAAAT	TACCGCAACG	ATCCCGACAG	GAAAGAGGGG
781	TTCTATGGTG	CAGGAGAAGT	AAACTGCGAG	TTTTGGGACT	CCGAACAAAA	CAGGAACAAG
841	TACATCTTAG	AACGAACTGG	AATCGCCATG	ACAAATTACA	ATTATGACAA	CTATAACTAC
901	AACCAGTCAG	ATCTTATTGC	TCCAGGATAT	CCTTCCGACC	CGAACTTCTA	CATTCCCATG
961	TATTTTGCAG	CACCTTGGGT	AGTTGTTAAG	GGATGCAGTG	GCAACAGCGA	TGAACAGTAC
1021	TCGTACGGAT	GGTTTATGGA	TAATGTCTCC	CAAACTTACA	TGAATACTGG	TGGTACTTCC
1081	TGGAAGTGTG	GAGAGGAGAA	CTTGGCATA	ATGGGAGCAC	AGTGCGGTCC	ATTTGACCAA
1141	CATTTTGTGT	ATGGTGATGG	AGATGGTCTT	GAGGATGTTG	TCCAAGCGTT	CTCTCTTCTG
1201	CAAGGCAAAG	AGTTTGAGAA	CCAAGTTCTG	AACAAACGTG	CCGTAATGCC	TCCGAAATAT
1261	GTGTTTGGTT	ACTTTCAGGG	AGTCTTTGGG	ATTGCTTCCT	TGTTGAGAGA	GCAAGAGCCA
1321	GAGGGTGGTA	ATAACATCTC	TGTTCAAGAG	ATTGTGGAAG	GTTACCAAAG	CAATAACTTC
1381	CCTTTAGAGG	GGTTAGCCGT	AGATGTGGAT	ATGCAACAAG	ATTTGCGCGT	GTTCAACCAG
1441	AAGATTGAAT	TTTGGACGGC	AAATAAGGTA	GGCACCAGGG	GAGACTCGAA	TAACAAGTCG
1501	GTGTTTGAAT	GGGCACATGA	CAAAGGCCCT	GTATGTCAGA	CGAATGTTAC	TTGCTTCTTG
1561	AGAAACGACA	ACGGCGGGGC	AGATTACGAA	GTCAATCAGA	CATTGAGGGA	GAAGGGTTTG
1621	TACACGAAGA	ATGACTCACT	GACGAACACT	AACTTCGGAA	CTACCAACGA	CGGGCCGAGC
1681	GATGCGTACA	TTGGACATCT	GGACTATGGT	GGCGGAGGGA	ATTGTGATGC	ACTTTTCCCA
1741	GACTGGGGTC	GACCGGGTGT	GGCTGAATGG	TGGGGTGATA	ACTACAGCAA	GCTCTTCAAA
1801	ATTGGTCTGG	ATTTCTGTCTG	GCAAGACATG	ACAGTTCCAG	CTATGATGCC	ACACAAAGTT
1861	GGCGACGCAG	TCGATACGAG	ATCACCTTAC	GGCTGGCCGA	ATGAGAATGA	TCCTTGAAC
1921	GGACGATACA	ATTGGAAATC	TTACCATCCA	CAAGTTCTCG	TAAGTGATAT	GCGATATGAG
1981	AATCATGGAA	GGGAACCGAT	GTTCACTCAA	CGCAATATGC	ATGCGTACAC	ACTCTGTGAA
2041	TCTACGAGGA	AGGAAGGGAT	TGTTGCAAA	GCAGACACTC	TAACGAAGTT	CGGCCGAGT
2101	TATATTATCA	GTCGTGGAGG	TTACATTGGC	AACCAGCATT	TTGGAGGAAT	GTGGGTTGGA
2161	GACAACTCTT	CCTCCCAAAG	ATACCTCCAA	ATGATGATCG	CGAACATCGT	CAACATGAAC
2221	ATGCTTGCC	TTCCACTAGT	TGGGTCCGAC	ATTGGAGGTT	TTACTTCGTA	TGATGACGSA
2281	AACGTGTGTC	CCGGGGATCT	AATGCTAAGA	TTGCTGCAGG	CGGGTTGCTT	ACTACCGTGG
2341	TTGAGAAACC	ACTATGGTAG	GTTGGTCCGAG	GGCAAGCAAG	AGGGAAAATA	CTATCAAGAA
2401	CTGTACATGT	ACAAGGACGA	GATGGCTACA	TTGAGAAAAT	TCATTGAATT	CCGTTACCGC
2461	TGGCAGGAGG	TGTTGTACAC	TGCTATGTAC	CAGAATGCGG	CTTTCGGGAA	ACCGATTATC
2521	AAGGCAGCTT	CCATGTACGA	CAACGACAGA	AACGTTGCGG	GCGCACAGGA	TGACCACTTC
2581	CTTCTCGGCG	GACACGATGG	ATATCGTAT	TTGTGTGCAC	CTGTTGTGTS	GGAGAATACA

FIGURE 8 CONTINUED

2641	ACCAGTCGCG	ATCTGTACTT	GCCTGTGCTG	ACCAAATGGT	ACAAATTCGG	CCCTGACTAT
2701	GACACCAAGC	GCCTGGATTG	TGCGTTGGAT	GGAGGGCAGA	TGATTAAGAA	CTATTCTGTG
2761	CCACAAAGCG	ACTCTCCGAT	ATTTGTGAGG	GAAGGAGCTA	TTCTCCCTAC	CCGCTACACG
2821	TTGGACGGTT	CGAACAAGTC	AATGAACACG	TACACAGACA	AAGACCCGTT	GGTGTTTGAG
2881	GTATTCCCTC	TTGGAAACAA	CCGTGCCGAC	GGTATGTGTT	ATCTTGATGA	TGGCGGTATT
2941	ACTACAGATG	CTGAGGACCA	TGGCAAATTC	TCTGTTATCA	ATGTCGAAGC	CTTACGGAAA
3001	GGTGTTACGA	CGACGATCAA	GTTTGCGTAT	GACACTTATC	AATACGTATT	TGATGGTCCA
3061	TTCTACGTTG	GAATCCGTAA	TCTTACGACT	GCATCAAAAA	TTAACGTGTC	TTCTGGAGCG
3121	GGTGAAGAGG	ACATGACACC	GACCTCTGCG	AACTCGAGGG	CAGCTTTGTT	CAGTGATGGA
3181	GGTGTTGGAG	AATACTGGGC	TGACAATGAT	ACGTCTTCTC	TGTGGATGAA	GTTGCCAAAC
3241	CTGGTTCCTG	AAGACGCTGT	GATTACCATT	ACGTAG		

FIGURE 9

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α -GLUCAN LYASE CODING SEQUENCE
 SEQUENCE TYPE: NUCLEIC ACID
 MOLECULE TYPE: DNA (GENOMIC)
 ORIGINAL SOURCE: FUNGUS
 SEQUENCE LENGTH: 3201 BP
 STRANDEDNESS: DOUBLE
 SEQUENCE.

10	20	30	40	50	60
ATGGCAGGAT	TTTCTGATCC	TCTCAACTTT	TGCAAAGCAG	AAGACTACTA	CAGTGTTCGG
70	80	90	100	110	120
CTAGACTGGA	AGGGCCCTCA	AAAAATCATT	GGAGTAGACA	CTACTCCTCC	AAAGAGCACC
130	140	150	160	170	180
AAGTTCCCCA	AAAAGTGGCA	TGGAGTGAAC	TTGAGATTCC	ATGATGGGAC	TTTAGGTGTG
190	200	210	220	230	240
GTTTCAGTTCA	TTAGGCCGTG	CGTTTGGAGG	GTTAGATACG	ACCCTGGTTT	CAAGACCTCT
250	260	270	280	290	300
GACGAGTATG	GTGATGAGAA	TACGAGGACA	ATTGTGCAAG	ATTATATGAG	TACTCTGAGT
310	320	330	340	350	360
AATAAATTGG	ATACTTATAG	AGGTCTTACG	TGGGAAACCA	AGTGTGAGGA	TTCGGGAGAT
370	380	390	400	410	420
TTCTTTACCT	TCTCATCCAA	GGTCACCGCC	GTTGAAAAAT	CCGAGCGGAC	CCGCAACAAG
430	440	450	460	470	480
GTCGGCGATG	GCCTCAGAAT	TCACCTATGG	AAAAGCCCTT	TCCGCATCCA	AGTAGTGCGC
490	500	510	520	530	540
ACCTTGACCC	CTTTGAAGGA	TCCTTACCCC	ATTCCAAATG	TAGCCGCAGC	CGAAGCCCGT
550	560	570	580	590	600
GTGTCCGACA	AGGTCGTTTG	GCAAACGTCT	CCCAAGACAT	TCAGAAAGAA	CCTGCATCCG
610	620	630	640	650	660
CAACACAAGA	TGCTAAAGGA	TACAGTTCTT	GACATTGTCA	AACCTGGACA	TGGCGAGTAT
670	680	690	700	710	720
GTGGGGTGGG	GAGAGATGGG	AGGTATCCAG	TTTATGAAGG	AGCCAACATT	CATGAACTAT
730	740	750	760	770	780
TTTAACTTCG	ACAATATGCA	ATACCAGCAA	GTCTATGCCC	AAGGTGCTCT	CGATTCTCGC
790	800	810	820	830	840
GAGCCACTGT	ACCACTCGGA	TCCCTTCTAT	CTTGATGTGA	ACTCCAACCC	GGAGCACAAAG
850	860	870	880	890	900
AATATCACGG	CAACCTTTAT	CGATAACTAC	TCTCAAATTG	CCATCGACTT	TGGAAAGACC
910	920	930	940	950	960
AACTCAGGCT	ACATCAAGCT	GGGAACCAGG	TATGGTGGTA	TCGATTGTTA	CGGTATCAGT
970	980	990	1000	1010	1020
GCGGATACGG	TCCCGGAAAT	TGTACGACTT	TATACAGGTC	TTGTTGGACG	TTCAAAGTTG
1030	1040	1050	1060	1070	1080
AAGCCCAGAT	ATATTCTGGG	GGCCCATCAA	GGCTGTTATG	GATACCAACA	GGAAAGTGAC
1090	1100	1110	1120	1130	1140
TTGTATTCTG	TGGTCCAGCA	GTACCGTGAC	TGTAAATTTG	CACCTTGACG	GATTTCACGTG
1150	1160	1170	1180	1190	1200
GATGTGCGATG	TTGAGGACGG	CTTCAGAACT	TTGACCAACA	ACCCACACAC	TTTCCCTAAC
1210	1220	1230	1240	1250	1260
CCCAAAGAGA	TGTTTACTAA	CTTGAGGAAT	AATGGAATCA	AGTGCTCCAC	CAATATCACT
1270	1280	1290	1300	1310	1320
CCTGTTATCA	GCATTAAACA	CAGAGAGGGT	GGATACAGTA	CCCTCCTTGA	GGGAGTTGAC

FIGURE 9 CONTINUED

1330	1340	1350	1360	1370	1380
AAAAAATACT	TTATCATGGA	CGACAGATAT	ACCGAGGGAA	CAAGTGGGAA	TGCGAAGGAT
1390	1400	1410	1420	1430	1440
GTTCCGTACA	TGTACTACGG	TGGTGGTAAT	AAGGTTGAGG	TCGATCCTAA	TGATGTTAAT
1450	1460	1470	1480	1490	1500
GGTCGCGCCAG	ACTTTAAAGA	CAACTATGAC	TTCCCCGCGA	ACTTCAACAG	CAAAACAATAC
1510	1520	1530	1540	1550	1560
CCCTATCATG	GTGGTGTGAG	CTACGGTTAT	GGGAACGGTA	GTGCAGGTTT	TTACCCGAGAC
1570	1580	1590	1600	1610	1620
CTCAACAGAA	AGGAGGTTCT	TATCTGGTGG	GGAATGCAGT	ACAAGTATCT	CTTCGATATG
1630	1640	1650	1660	1670	1680
GGACTGGAAT	TTGTGTGGCA	AGACATGACT	ACCCCAGCAA	TCCACACATC	ATATGGAGAC
1690	1700	1710	1720	1730	1740
ATGAAAGGGT	TGCCACCCCG	TCTACTCGTC	ACCTCAGACT	CCGTCACCAA	TGCTCTGAG
1750	1760	1770	1780	1790	1800
AAAAAGCTCG	CAATTGAAAC	TTGGGCTCTC	TACTCCTACA	ATCTCCACAA	AGCAACTTGG
1810	1820	1830	1840	1850	1860
CATGGTCTTA	GTCGTCTCGA	ATCTCGTAAG	AACAAACGAA	ACTTCATCCT	CGGGCGTGGA
1870	1880	1890	1900	1910	1920
AGTTATGCCG	GAGCCTATCG	TTTTGCTGGT	CTCTGGACTG	GGGATAATGC	AAGTAACTGG
1930	1940	1950	1960	1970	1980
GAATTCTGGA	AGATATCGGT	CTCTCAAGTT	CTTTCTCTGG	GCCTCAATGG	TGTGTGCATC
1990	2000	2010	2020	2030	2040
GCGGGGTCTG	ATACGGGTGG	TTTTGAACCC	TACCGTGATG	CAAATGGGGT	CGAGGAGAAA
2050	2060	2070	2080	2090	2100
TACTGTAGCC	CAGAGCTACT	CATCAGGTGG	TATACTGGTT	CATTCTCTTT	GCCGTGGCTC
2110	2120	2130	2140	2150	2160
AGGAACCAAT	ATGTCAAAAA	GGACAGGAAA	TGGTTCCAGG	AACCATACTC	GTACCCCAAG
2170	2180	2190	2200	2210	2220
CATCTTGAAA	CCCATCCAGA	ACTCGCAGAC	CAAGCATGGC	TCTATAAATC	CGTTTTGGAG
2230	2240	2250	2260	2270	2280
ATCTGTAGGT	ACTATGTGGA	GCTTAGATAC	TCCCTCATCC	AACTACTTTA	CGACTGCATG
2290	2300	2310	2320	2330	2340
TTTCAAAACG	TaGTCGACGG	TATGCCAATC	ACCAGATCTA	TGCTCTTGAC	CGATACTGAG
2350	2360	2370	2380	2390	2400
GATACCACCT	TCTTCAACGA	GAGCCAAAAG	TTCCCTCGACA	ACCAATATAT	GGCTGGTGAC
2410	2420	2430	2440	2450	2460
GACATTCTTG	TTGCACCCAT	CCTCCACAGT	CGCAAAGAAA	TTCCAGGCGA	AAACAGAGAT
2470	2480	2490	2500	2510	2520
GTCTATCTCC	CTCTTTACCA	CACCTGGTAC	CCCTCAAATT	TGAGACCATG	GGACGATCAA
2530	2540	2550	2560	2570	2580
GGAGTCGCTT	TGGGGAATCC	TGTCGAAGGT	GGTAGTGTCA	TCAATTATAC	TGCTAGGATT
2590	2600	2610	2620	2630	2640
GTTGCACCCG	AGGATTATAA	TCTCTTCCAC	AGCGTGGTAC	CAGTCTACGT	TAGAGAGGGT
2650	2660	2670	2680	2690	2700
GCCATCATCC	CGCAAATCGA	AGTACGCCAA	TGGACTGGCC	AGGGGGGAGC	CAACCGCATC
2710	2720	2730	2740	2750	2760
AAGTTCAACA	TCTACCCCTG	AAAGGATAAG	GAGTACTGTA	CCTATCTTGA	TGATGSGTGT
2770	2780	2790	2800	2810	2820
AGCCGTGATA	GTGCGCCGGA	AGACCTCCCA	CAGTACAAAG	AGACCCACGA	ACAGTCGAAG
2830	2840	2850	2860	2870	2880
GTTGAAGGCG	CGGAAATCGC	AAAGCAGATT	GGAAAGAAGA	CGGGTTACAA	CATCTCAGGA
2890	2900	2910	2920	2930	2940

FIGURE 9 CONTINUED

ACCGACCCAG	AAGCAAAGGG	TTATCACCGC	AAAGTTGCTG	TCACACAAAC	GTCAAAAGAC
2950	2960	2970	2980	2990	3000
AAGACGCGTA	CTGTCACTAT	TGAGCCAAAA	CACAATGGAT	ACGACCCTTC	CAAAGAGGTG
3010	3020	3030	3040	3050	3060
GGTGATTATT	ATACCATCAT	TCTTTGGTAC	GCACCAGGTT	TCGATGGCAG	CATCGTCGAT
3070	3080	3090	3100	3110	3120
GTGAGCAAGA	CGACTGTGAA	TGTTGAGGGT	GGGGTGGAGC	ACCAAGTTTA	TAAGAACTCC
3130	3140	3150	3160	3170	3180
GATTACATA	CGGTTGTTAT	CGACGTGAAG	GAGGTGATCG	GTACCACAAA	GAGCGTCAAG
3190	3200				
ATCACATGTA	CTGCCGCTTA	A			

FIGURE 10

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α -GLUCAN LYASE CODING SEQUENCE
 SEQUENCE TYPE: NUCLEIC ACID
 MOLECULE TYPE: DNA (GENOMIC)
 ORIGINAL SOURCE: FUNGUS
 SEQUENCE LENGTH: 3213 BP
 STRANDEDNESS: DOUBLE
 SEQUENCE:

10	20	30	40	50	60
ATGGCAGGAT	TATCCGACCC	TCTCAATTTG	TGCAAAGCAG	AGGACTACTA	CGCTGCTGCC
70	80	90	100	110	120
AAAGGCTGGA	GTGGCCCTCA	GAAGATCATT	CGCTATGACC	AGACCCCTCC	TCAGGGTACA
130	140	150	160	170	180
AAAGATCCGA	AAAGCTGGCA	TGCGGTAAAC	CTTCCTTTCC	ATGACGGGAC	TATGTGTGTA
190	200	210	220	230	240
GTGCAATTCG	TCAGACCCTG	TGTTTGGAGG	GTTAGATATG	ACCCCACTGT	CAAGACTTCT
250	260	270	280	290	300
GATGAGTACG	GCGATGAGAA	TACGAGGACT	ATTGTACAAG	ACTACATGAC	TACTCTGGTT
310	320	330	340	350	360
GGAAACTTGG	ACATTTTCAG	AGGTCTTACG	TGGGTTTCTA	CGTTGGAGGA	TTCGGGCGAG
370	380	390	400	410	420
TACTACACCT	TCAAGTCCGA	AGTCACTGCC	GTGGACGAAA	CCGAACGGAC	TCGAAACAAG
430	440	450	460	470	480
GTCGGCGACG	GCCTCAAGAT	TTACCTATGG	AAAAATCCCT	TTCGCATCCA	GGTAGTGCGT
490	500	510	520	530	540
CTCTTGACCC	CCCTGGTGGA	CCCTTTCCCC	ATTCCCAACG	TAGCCAATGC	CACAGCCCGT
550	560	570	580	590	600
GTGGCCGACA	AGGTTGTTTG	GCAGACGTCC	CCGAAGACGT	TCAGGAAAAA	CTTGCATCCG
610	620	630	640	650	660
CAGCATAAGA	TGTTGAAGGA	TACAGTTCTT	GATATTATCA	AGCCGGGGCA	CGGAGAGTAT
670	680	690	700	710	720
GTGGGTTGGG	GAGAGATGGG	AGGCATCGAG	TTTATGAAGG	AGCCAACATT	CATGAATTAT
730	740	750	760	770	780
TTCAACTTTG	ACAATATGCA	ATATCAGCAG	GTCTATGCAC	AAGGCGCTCT	TGATAGTCGT
790	800	810	820	830	840
GAGCCGTTGT	ATCACTCTGA	TCCCTTCTAT	CTCGACGTGA	ACTCCAACCC	AGAGCACAAG
850	860	870	880	890	900
AACATTACGG	CAACCTTTAT	CGATAACTAC	TCTCAGATTG	CCATCGACTT	TGGGAAGACC
910	920	930	940	950	960
AACTCAGGCT	ACATCAAGCT	GGGTACCAGG	TATGGCGGTA	TGGATTGTTA	CGGTATCAGC
970	980	990	1000	1010	1020
GCGGATACGG	TCCCGGAGAT	TGTGCGACTT	TATACTGGAC	TTGTTGGGCG	TTCGAAGTTG
1030	1040	1050	1060	1070	1080
AAGCCGAGGT	ATATCTCTCG	AGCCCACCAA	GCCTGTATATG	GATACCAGCA	GGAAAGTGAC
1090	1100	1110	1120	1130	1140
TTGCATGCTG	TTGTTCAGCA	GTACCGTGAC	ACCAAGTTTC	CGCTTGATGG	GTTGCATGTC
1150	1160	1170	1180	1190	1200
GATGTGCACT	TTGAGGACAA	TTTCAGAACG	TTTACCACTA	ACCGGATTAC	GTTCCCTAAT
1210	1220	1230	1240	1250	1260
CCCAAAGAAA	TGTTTACCAA	TCTAAGGAAAC	AATGGAAATCA	AGTGTTCACAC	CAACATCACC
1270	1280	1290	1300	1310	1320

FIGURE 10 CONTINUED

CCTGTTATCA	GTATCAGAGA	TCGCCCCGAAT	GGGTACAGTA	CCCTCAATGA	GGGATATGAT
1330	1340	1350	1360	1370	1380
AAAAAGTACT	TCATCATGGA	TGACAGATAT	ACCGAGGGGA	CAAGTGGGGA	CCCGCAAAAT
1390	1400	1410	1420	1430	1440
GTTTCGATACT	CTTTTTACGG	CGGTGGGAAC	CCGGTTGAGG	TTAACCCCTAA	TGATGTTTGG
1450	1460	1470	1480	1490	1500
GCTCGGCCAG	ACTTTGGAGA	CAATTATGAC	TTCCCTACGA	ACTTCAACTG	CAAAGACTAC
1510	1520	1530	1540	1550	1560
CCCTATCATG	GTGGTGTGAG	TTACGGATAT	GGGAATGGCA	CTCCAGGTTA	CTACCCGTGAC
1570	1580	1590	1600	1610	1620
CTTAACAGAG	AGGAGGTTCC	TATCTGGTGG	GGATTGCAGT	ACGAGTATCT	CTTCAATATC
1630	1640	1650	1660	1670	1680
GGACTAGAGT	TTGTATGGCA	AGATATGACA	ACCCCAGCGA	TCCATTTCATC	ATATGGAGAC
1690	1700	1710	1720	1730	1740
ATGAAAGGGT	TGCCCCACCG	TCTGCTCGTC	ACCGCCGACT	CAGTTACCAA	TGCCTCTGAG
1750	1760	1770	1780	1790	1800
AAAAAGCTCG	CAATTGAAAG	TTGGGCTCTT	TACTCCTACA	ACCTCCATAA	AGCAACCTTC
1810	1820	1830	1840	1850	1860
CACGGTCTTG	GTCGTCTTGA	GTCTCGTAAG	AACAAACGTA	ACTTCATCCT	CGGACGTGGT
1870	1880	1890	1900	1910	1920
AGTTACGCCG	GTGCCTATCG	TTTTGCTGGT	CTCTGGACTG	GAGATAACGC	AAGTACGTGG
1930	1940	1950	1960	1970	1980
GAATTCTGGA	AGATTTCCGT	CTCCCAAGTT	CTTTCTCTAG	GTCTCAATGG	TGTGTGTATA
1990	2000	2010	2020	2030	2040
GCGGGGTCTG	ATACGGGTGG	TTTTGAGCCC	GCACGTACTG	AGATTGGGGA	GGAGAAATAT
2050	2060	2070	2080	2090	2100
TGCAGTCCGG	AGCTACTCAT	CAGGTGGTAT	ACTGGATCAT	TCCTTTTGCC	ATGGCTTAGA
2110	2120	2130	2140	2150	2160
AACCACTACG	TCAAGAAGGA	CAGGAAATGG	TTCCAGGAAC	CATACGCGTA	CCCCAAGCAT
2170	2180	2190	2200	2210	2220
CTTGAAACCC	ATCCAGAGCT	CGCAGATCAA	GCATGGCTTT	ACAAATCTGT	TCTAGAAATT
2230	2240	2250	2260	2270	2280
TGCAGATACT	GGGTAGAGCT	AAGATATTCC	CTCATCCAGC	TCCTTTACGA	CTGCATGTTT
2290	2300	2310	2320	2330	2340
CAAAACGTGG	TCGATGGTAT	GCCACTTGCC	AGATCTATGC	TCTTGACCGA	TACTGAGGAT
2350	2360	2370	2380	2390	2400
ACGACCTTCT	TCAATGAGAG	CCAAAAGTTC	CTCGATAACC	AATATATGGC	TGGTGACGAC
2410	2420	2430	2440	2450	2460
ATCCTTGTAG	CACCCATCCT	CCACAGCCGT	AACGAGGTTC	CGGGAGAGAA	CAGAGATGTC
2470	2480	2490	2500	2510	2520
TATCTCCCTC	TATCCACAC	CTGGTACCCC	TCAAACCTGA	GACCGTGGGA	CGATCAGGGA
2530	2540	2550	2560	2570	2580
GTCGCTTTAG	GGAATCCTGT	CGAAGGTGGC	AGCGTTATCA	ACTACACTGC	CAGGATTGTT
2590	2600	2610	2620	2630	2640
GCCCCAGAGG	ATTATAATCT	CTTCCACAAC	GTGGTGCCGG	TCTACATCAG	AGAGGGTGCC
2650	2660	2670	2680	2690	2700
ATCATTCCGC	AAATTCAGGT	ACGCCAGTGG	ATTGGCGAAG	GAGGGCCCTAA	TCCCATCAAG
2710	2720	2730	2740	2750	2760
TTCAATATCT	ACCCTGGAAA	GGACAAGGAG	TATGTGACGT	ACCTTGATGA	TGGTGTAGC
2770	2780	2790	2800	2810	2820
CGCGATAGTG	CACCAGATGA	CCTCCCGCAG	TACCGCGAGG	CCTATGAGCA	AGCGAAGGTC
2830	2840	2850	2860	2870	2880

FIGURE 10 CONTINUED

GAAGGCAAAG	ACGTCCAGAA	GCAACTTGCG	GTCATTCAAG	GGAATAAGAC	TAATGACTTC
2890	2900	2910	2920	2930	2940
TCCGCCTCCG	GGATTGATAA	GGAGGCAAAG	GGTTATCACC	GCAAAGTTTC	TATCAAACAG
2950	2960	2970	2980	2990	3000
GAGTCAAAAG	ACAAGACCCG	TACTGTCACC	ATTGAGCCAA	AACACAACGG	ATACGACCCC
3010	3020	3030	3040	3050	3060
TCTAAGGAAG	TTGGTAATTA	TTATACCATC	ATTCTTTGGT	ACGCACCGGG	CTTGACGGC
3070	3080	3090	3100	3110	3120
AGCATCGTCG	ATGTGAGCCA	GGCGACCGTG	AACATCGAGG	GCGGGGTGGA	ATGCGAAATT
3130	3140	3150	3160	3170	3180
TTCAAGAACA	CCGGCTTGCA	TACGGTTGTA	GTCAACGTGA	AAGAGGTGAT	CGGTACCACA
3190	3200	3210			
AAGTCCGTCA	AGATCACTTG	CACTACCGCT	TAG		

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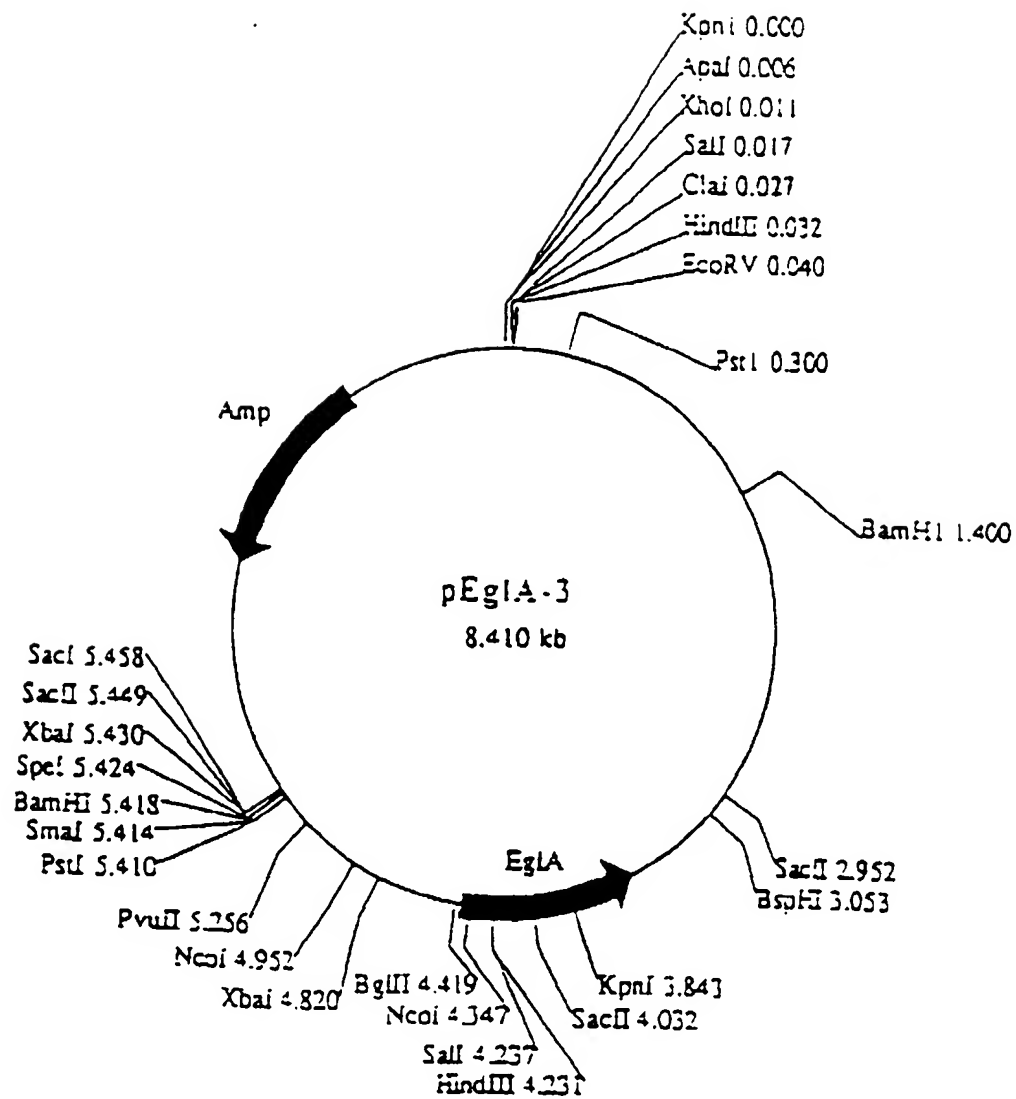


FIG. 11

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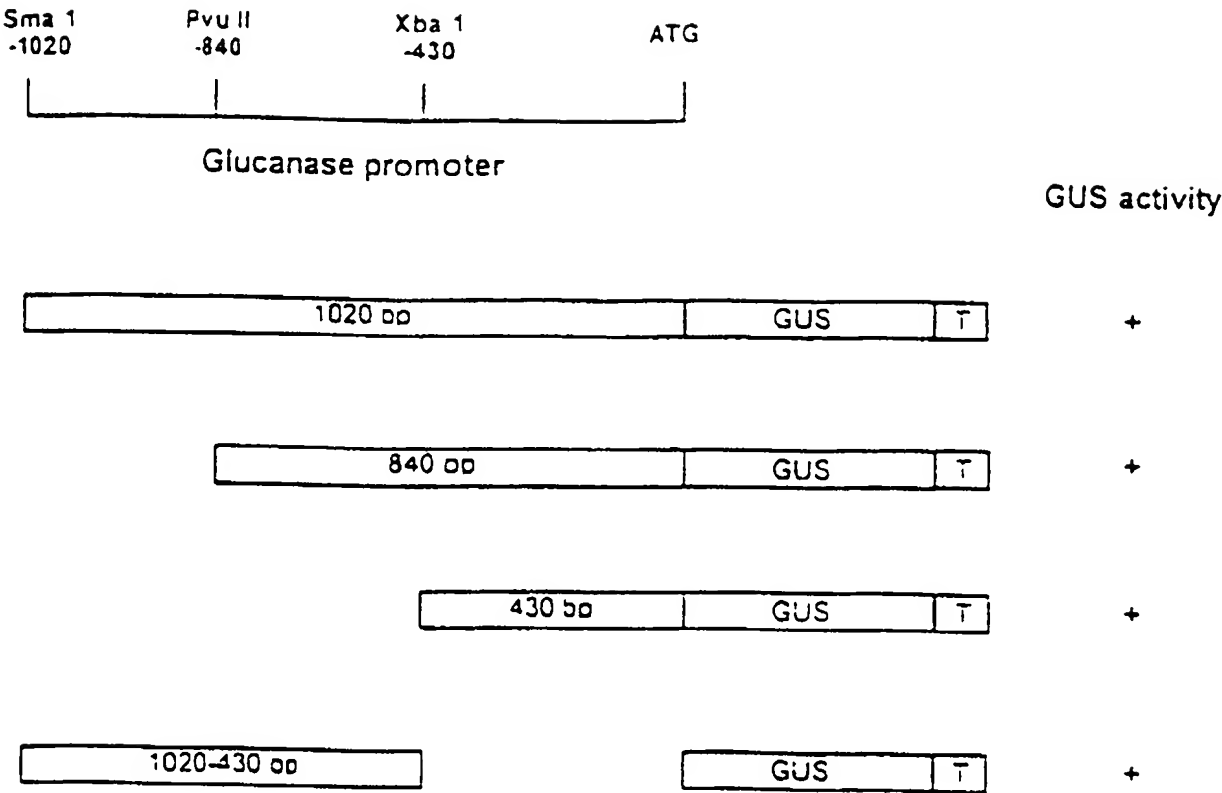


FIG. 12

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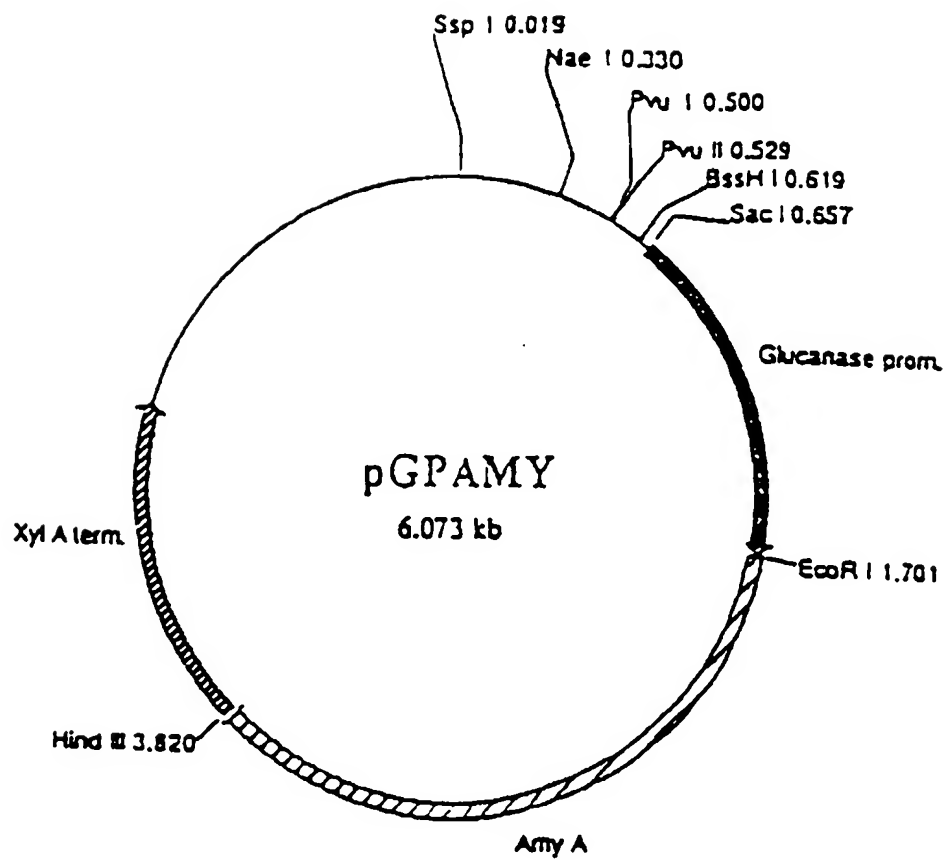


FIG. 13

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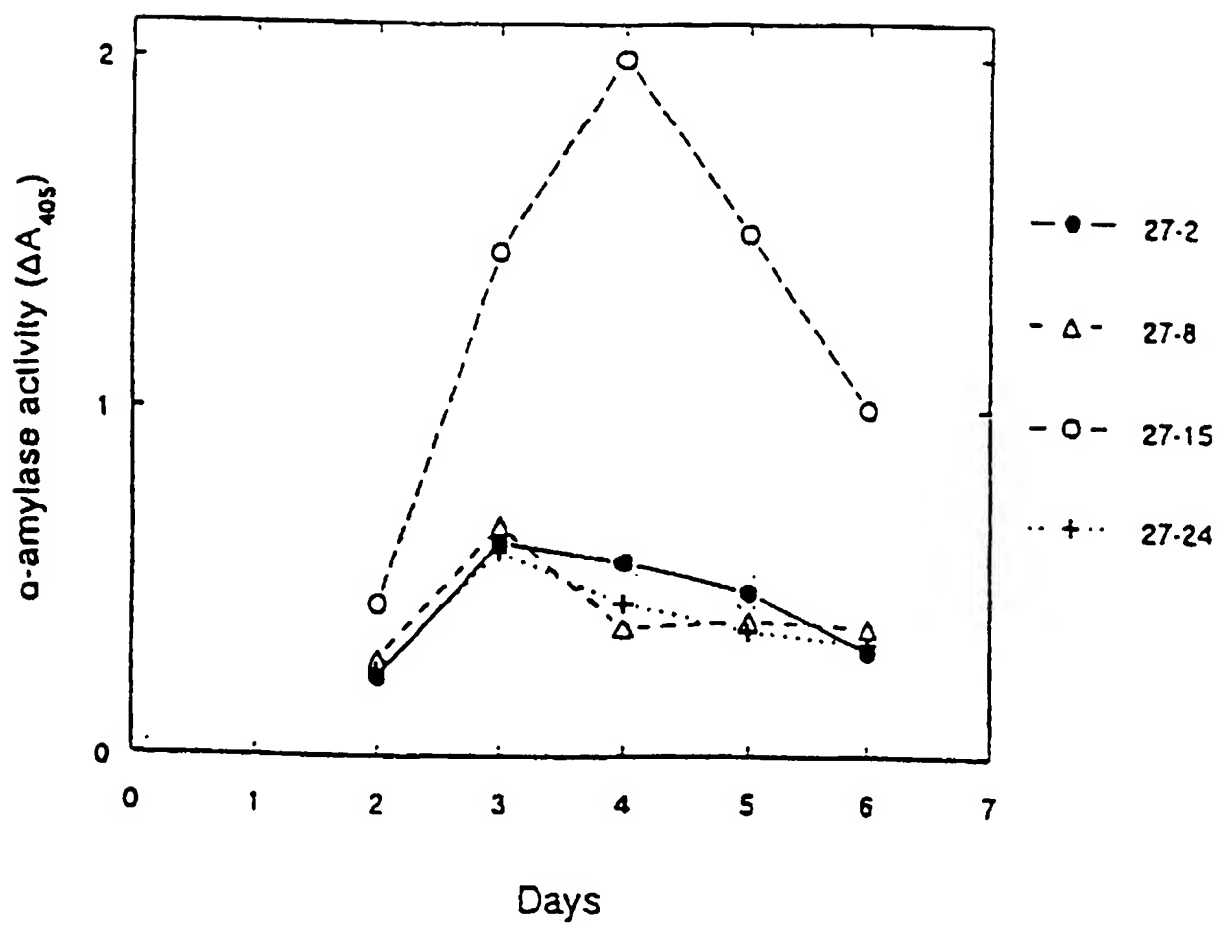


FIG. 14

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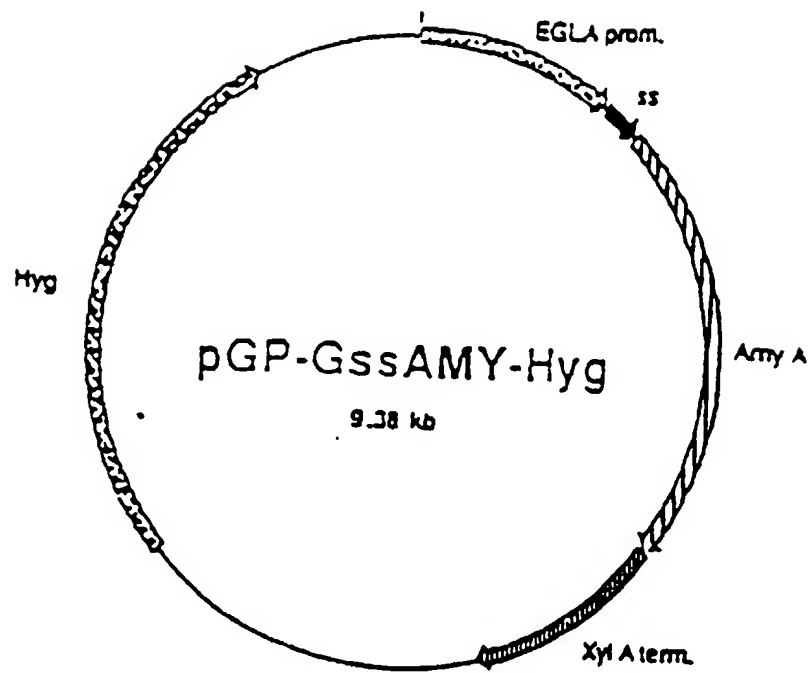


FIG. 15

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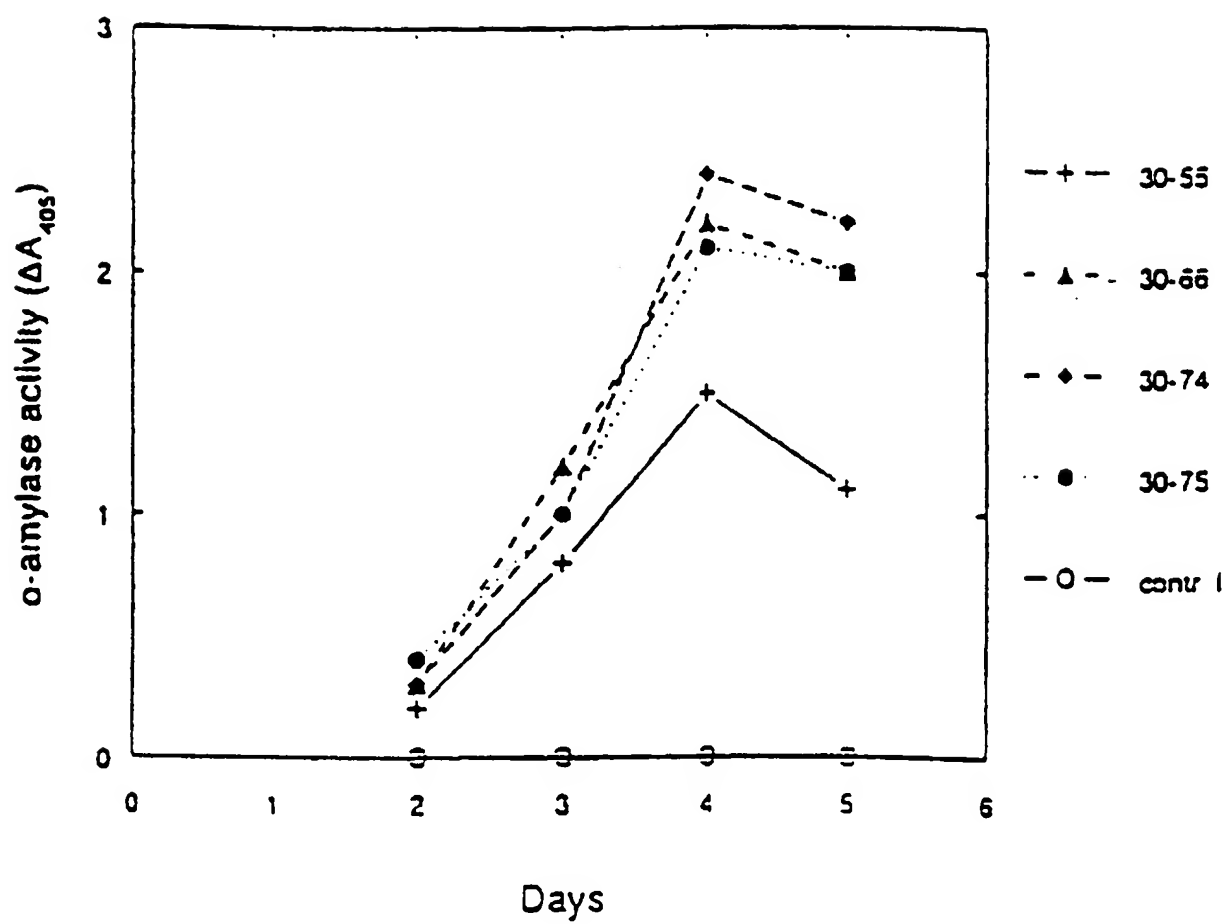


FIG. 16

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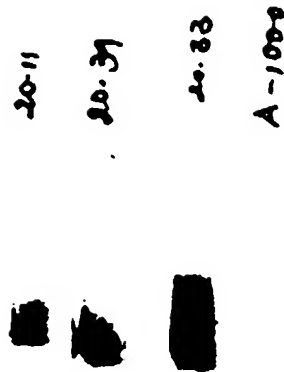


FIG. 17

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 96/01008

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/56 C12N9/42 C12N15/80 C12N15/62 C12N1/15
 C12N1/19 C12N5/10 //(C12N1/15,C12R1:66)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL EMFUN:SCD12901;ACCES-NO:D12901 SAKAMOTO,S. ET AL. Cloning and sequencing of the cellulase XP002009466 cDNA from <i>Aspergillus kawachii</i> and its expression in <i>Saccharomyces cerevisiae</i> . 13aug1992; abstr.	1-9, 13-18, 21-24, 26,28,29
Y	EP,A,0 458 162 (KAO CORPORATION) 27 November 1991 see claims	1

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

A document member of the same patent family

Date of the actual completion of the international search

29 July 1996

Date of mailing of the international search report

07. 08. 96

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 Fax (+ 31-70) 340-3016

Authorized officer

Delanghe, L

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 96/01008

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NUCLEIC ACIDS RESEARCH, vol. 18, no. 19, 11 October 1990, OXFORD GB, page 5884 XP002009463 TOSHIHIKO OOI ET AL.: "Complete nucleotide sequence of a gene coding for Aspergillus aculeatus cellulase (FI-CMCase)" see the whole document ---	1
Y	AGRICULTURAL AND BIOLOGICAL CHEMISTRY, vol. 49, no. 5, May 1985, TOKYO JP, pages 1257-1265, XP002009464 GENTARO OKADA: "Purification and properties of a cellulase from Aspergillus niger" see the whole document ---	1
P,X	CURRENT GENETICS, vol. 27, no. 5, April 1995, pages 435-439, XP002009465 S.SAKAMOTO ET AL.: "Cloning and sequencing of cellulase cDNA from Aspergillus kawachii and its expression in Saccharomyces cerevisiae" see the whole document -----	1-9, 13-18, 21-24, 26,28,29

Information on patent family members

PLI/EP 96/01008

Form PCT ISA 210 (patent family annex) (July 1992)